Supporting Information for

High-dimensional solid-state NMR facilitated by transverse-mixing optimal control

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Table of Contents

Sensitivity enhancement for the 2-fold enhanced HNcoCANH at 800 MHz

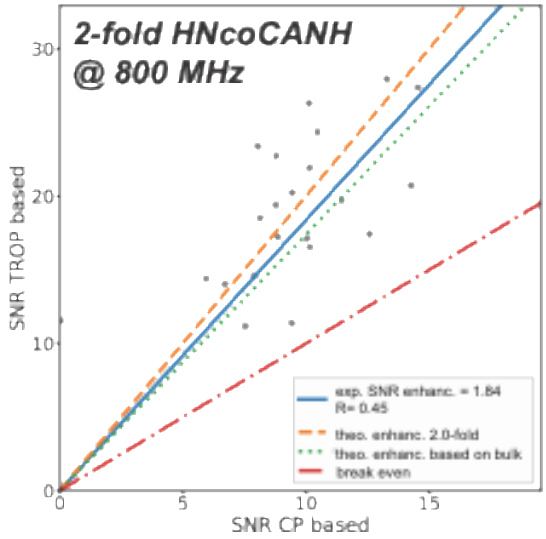

Fig. S1: Sensitivity enhancements found for the doubly enhanced 5D HNcoCANH experiment recorded at 800 MHz Larmor frequency, after reconstruction. The enhancement is close to the expected factors based on bulk signal attenuation, as here the sensitivity-enhanced experiment features slightly lower bulk intensity compared to the CP-based experiment (unavailability of the newest-generation NH TROP, see main text). Nevertheless, even in this case, improvements can be observed.

Bulk intensities for the first FID under various conditions

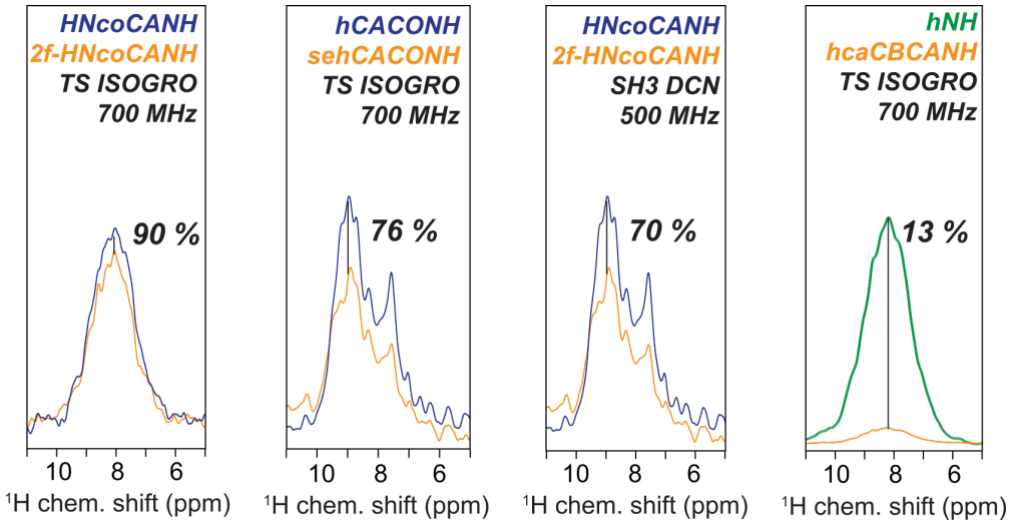

Fig. S2: Bulk sensitivities of the first FID for different fully and partially enhanced experiments recorded across different samples, labelling schemes, and magnetic field strength. The performance of experiments utilizing TROPs is largely preserved across different conditions. The rightmost case represents the bulk signal of the two-fold enhanced hcaCBCANH compared to the hNH, representing at the same time performance for a different (high-molecular-weight) protein.

Sensitivty enhancements for the 3-fold enhanced HNcoCANH using nuFT

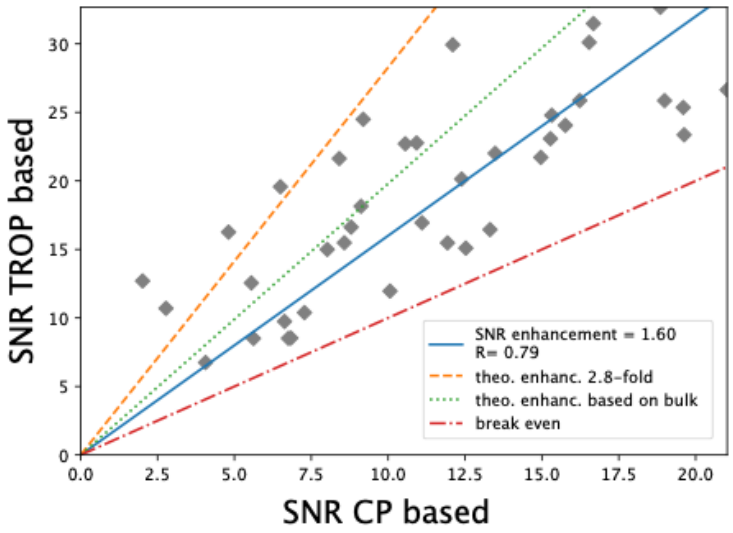

Fig. S3: Sensitivity enhancements found for the triply enhanced 5D HNcoCANH experiment recorded at 800 MHz, before reconstruction, i.e., using nuFT (unavailability of the newest-generation NH TROP). As expected, the enhancements are below the theoretical and expected factors based on bulk signal attenuation, as the sensitivity-enhanced experiment features an overall higher noise level compared to the CP based experiment (see above). Nevertheless, even in this case, a moderate improvement can be observed.

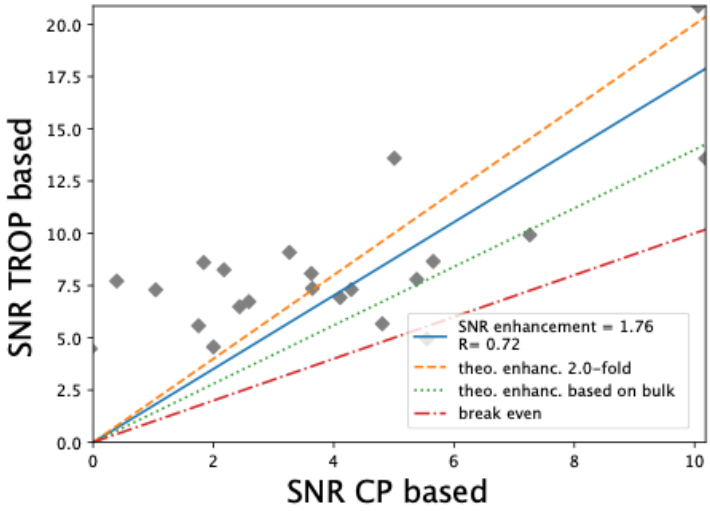

Fig. S4: Sensitivity enhancements found for the double enhanced 5D HNcoCANH experiment recorded at 500 MHz, before reconstruction, i.e., using nuFT (unavailability of the newest-generation NH TROP). As noise reduction was overall lower and close to zero for these data (see main text), the nuFT spectra show similar enhancement factors compared to the reconstructed spectrum, when using the same processing parameters. This is also reflected in the noise levels found for the individual 2D planes as shown above.

Combining sensitivity enhanced E/AE with traditional States

In this section, we elaborate time evolution of NMR signal during a 3D experiment where the two indirect domains are encoded using either the traditional States/TPPI or Echo-Antiecho approaches.

Assume three arrangements:
Scheme 1: - t1 (States) – mixing – t2 (E/AE) – mixing – t3 (acquisition)
Scheme 2: - t1 (E/AE) – mixing – t2 (States) – mixing – t3 (acquisition)
Scheme 3: - t1 (E/AE) – mixing – t2 (E/AE) – mixing – t3 (acquisition)

In **Scheme 1**, we acquire these four signals for each (t_1, t_2) pair:
FID1

$$\xrightarrow{t_1} I_x \cos\Omega_1 t_1 + I_y \sin\Omega_1 t_1 \xrightarrow{States\ x-mix} S_x \cos\Omega_1 t_1 \xrightarrow{t_2} S_x \cos\Omega_2 t_2 \cos\Omega_1 t_1 + S_y \sin\Omega_2 t_2 \cos\Omega_1 t_1 \xrightarrow{TROP\ E-mix} F_x \cos\Omega_2 t_2 \cos\Omega_1 t_1 + F_y \sin\Omega_2 t_2 \cos\Omega_1 t_1$$

FID2

$$\xrightarrow{t_1} I_x \cos\Omega_1 t_1 + I_y \sin\Omega_1 t_1 \xrightarrow{States\ x-mix} S_x \cos\Omega_1 t_1 \xrightarrow{t_2} S_x \cos\Omega_2 t_2 \cos\Omega_1 t_1 + S_y \sin\Omega_2 t_2 \cos\Omega_1 t_1 \xrightarrow{TROP\ AE-mix} F_x \cos\Omega_2 t_2 \cos\Omega_1 t_1 - F_y \sin\Omega_2 t_2 \cos\Omega_1 t_1$$

FID3

$$\xrightarrow{t_1} I_x \cos\Omega_1 t_1 + I_y \sin\Omega_1 t_1 \xrightarrow{States\ y-mix} S_y \sin\Omega_1 t_1 \xrightarrow{t_2} S_y \cos\Omega_2 t_2 \sin\Omega_1 t_1 - S_x \sin\Omega_2 t_2 \sin\Omega_1 t_1 \xrightarrow{TROP\ E-mix} F_y \cos\Omega_2 t_2 \sin\Omega_1 t_1 - F_x \sin\Omega_2 t_2 \sin\Omega_1 t_1$$

FID4

$$\xrightarrow{t_1} I_x \cos\Omega_1 t_1 + I_y \sin\Omega_1 t_1 \xrightarrow{States\ y-mix} S_y \sin\Omega_1 t_1 \xrightarrow{t_2} S_y \cos\Omega_2 t_2 \sin\Omega_1 t_1 - S_x \sin\Omega_2 t_2 \sin\Omega_1 t_1 \xrightarrow{TROP\ AE-mix} -F_y \cos\Omega_2 t_2 \sin\Omega_1 t_1 - F_x \sin\Omega_2 t_2 \sin\Omega_1 t_1$$

Reconstruction of cos and sin modulated data is performed according to the table below.
Σ_1= (FID1+FID2) = $2F_x \cos\Omega_2 t_2 \cos\Omega_1 t_1$
Δ_1= (FID1-FID2) = $2F_y \sin\Omega_2 t_2 \cos\Omega_1 t_1$
Σ_2= (FID3+FID4) = $-2F_x \sin\Omega_2 t_2 \sin\Omega_1 t_1$
Δ_2= (FID3-FID4) = $2F_y \cos\Omega_2 t_2 \sin\Omega_1 t_1$

		In t_1	
		cos	sin
In t_2	cos	Σ_1	Δ_2
	sin	Δ_1	Σ_2

In **Scheme 2**, we acquire these four signals for each (t_1, t_2) pair:
FID1

$$\xrightarrow{t_1} I_x \cos\Omega_1 t_1 + I_y \sin\Omega_1 t_1 \xrightarrow{TROP\ E-mix} S_x \cos\Omega_1 t_1 + S_y \sin\Omega_1 t_1 \xrightarrow{t_2} S_x \cos\Omega_2 t_2 \cos\Omega_1 t_1 + S_y \sin\Omega_2 t_2 \cos\Omega_1 t_1 + S_y \cos\Omega_2 t_2 \sin\Omega_1 t_1 - S_x \sin\Omega_2 t_2 \sin\Omega_1 t_1 \xrightarrow{States\ x-mix} F_x \cos\Omega_2 t_2 \cos\Omega_1 t_1 - F_x \sin\Omega_2 t_2 \sin\Omega_1 t_1$$

FID2

$$\xrightarrow{t_1} I_x \cos\Omega_1 t_1 + I_y \sin\Omega_1 t_1 \xrightarrow{TROP\ E-mix} S_x \cos\Omega_1 t_1 + S_y \sin\Omega_1 t_1 \xrightarrow{t_2} S_x \cos\Omega_2 t_2 \cos\Omega_1 t_1 + S_y \sin\Omega_2 t_2 \cos\Omega_1 t_1 + S_y \cos\Omega_2 t_2 \sin\Omega_1 t_1 - S_x \sin\Omega_2 t_2 \sin\Omega_1 t_1 \xrightarrow{States\ y-mix} F_y \sin\Omega_2 t_2 \cos\Omega_1 t_1 + F_y \cos\Omega_2 t_2 \sin\Omega_1 t_1$$

FID3

$$\xrightarrow{t_1} I_x \cos\Omega_1 t_1 + I_y \sin\Omega_1 t_1 \xrightarrow{TROP\ AE-mix} S_x \cos\Omega_1 t_1 - S_y \sin\Omega_1 t_1 \xrightarrow{t_2} S_x \cos\Omega_2 t_2 \cos\Omega_1 t_1 + S_y \sin\Omega_2 t_2 \cos\Omega_1 t_1 - S_y \cos\Omega_2 t_2 \sin\Omega_1 t_1 + S_x \sin\Omega_2 t_2 \sin\Omega_1 t_1 \xrightarrow{States\ x-mix} F_x \cos\Omega_2 t_2 \cos\Omega_1 t_1 + F_x \sin\Omega_2 t_2 \sin\Omega_1 t_1$$

FID4

$$\xrightarrow{t_1} I_x \cos\Omega_1 t_1 + I_y \sin\Omega_1 t_1 \xrightarrow{TROP\ AE-mix} S_x \cos\Omega_1 t_1 - S_y \sin\Omega_1 t_1 \xrightarrow{t_2} S_x \cos\Omega_2 t_2 \cos\Omega_1 t_1 + S_y \sin\Omega_2 t_2 \cos\Omega_1 t_1 - S_y \cos\Omega_2 t_2 \sin\Omega_1 t_1 + S_x \sin\Omega_2 t_2 \sin\Omega_1 t_1 \xrightarrow{States\ y-mix} F_y \sin\Omega_2 t_2 \cos\Omega_1 t_1 - F_y \cos\Omega_2 t_2 \sin\Omega_1 t_1$$

Reconstruction of cos and sin modulated data is performed according to the table below.

Σ_1= (FID1+FID3) = $2F_x \cos\Omega_2 t_2 \cos\Omega_1 t_1$

Δ_1= (FID1-FID3) = $2F_x \sin\Omega_2 t_2 \sin\Omega_1 t_1$

Σ_2= (FID2+FID4) = $2F_y \sin\Omega_2 t_2 \cos\Omega_1 t_1$

Δ_2= (FID2-FID4) = $2F_y \cos\Omega_2 t_2 \sin\Omega_1 t_1$

		In t_1	
		cos	sin
In t_2	cos	Σ_1	Δ_2
	sin	Σ_2	Δ_1

In **Scheme 3**, we acquire these four signals for each (t_1, t_2) pair:

FID1

$$\xrightarrow{t_1} I_x \cos\Omega_1 t_1 + I_y \sin\Omega_1 t_1 \xrightarrow{TROP\ E-mix} S_x \cos\Omega_1 t_1 + S_y \sin\Omega_1 t_1 \xrightarrow{t_2} S_x \cos\Omega_2 t_2 \cos\Omega_1 t_1 + S_y \sin\Omega_2 t_2 \cos\Omega_1 t_1 + S_y \cos\Omega_2 t_2 \sin\Omega_1 t_1 - S_x \sin\Omega_2 t_2 \sin\Omega_1 t_1 \xrightarrow{TROP\ E-mix} F_x \cos\Omega_2 t_2 \cos\Omega_1 t_1 + F_y \sin\Omega_2 t_2 \cos\Omega_1 t_1 + F_y \cos\Omega_2 t_2 \sin\Omega_1 t_1 - F_x \sin\Omega_2 t_2 \sin\Omega_1 t_1$$

FID2

$$\xrightarrow{t_1} I_x \cos\Omega_1 t_1 + I_y \sin\Omega_1 t_1 \xrightarrow{TROP\ E-mix} S_x \cos\Omega_1 t_1 + S_y \sin\Omega_1 t_1 \xrightarrow{t_2} S_x \cos\Omega_2 t_2 \cos\Omega_1 t_1 + S_y \sin\Omega_2 t_2 \cos\Omega_1 t_1 + S_y \cos\Omega_2 t_2 \sin\Omega_1 t_1 - S_x \sin\Omega_2 t_2 \sin\Omega_1 t_1 \xrightarrow{TROP\ AE-mix} F_x \cos\Omega_2 t_2 \cos\Omega_1 t_1 - F_y \sin\Omega_2 t_2 \cos\Omega_1 t_1 - F_y \cos\Omega_2 t_2 \sin\Omega_1 t_1 - F_x \sin\Omega_2 t_2 \sin\Omega_1 t_1$$

FID3

$$\xrightarrow{t_1} I_x \cos\Omega_1 t_1 + I_y \sin\Omega_1 t_1 \xrightarrow{TROP\ AE-mix} S_x \cos\Omega_1 t_1 - S_y \sin\Omega_1 t_1 \xrightarrow{t_2} S_x \cos\Omega_2 t_2 \cos\Omega_1 t_1 + S_y \sin\Omega_2 t_2 \cos\Omega_1 t_1 - S_y \cos\Omega_2 t_2 \sin\Omega_1 t_1 + S_x \sin\Omega_2 t_2 \sin\Omega_1 t_1 \xrightarrow{TROP\ E-mix} F_x \cos\Omega_2 t_2 \cos\Omega_1 t_1 + F_y \sin\Omega_2 t_2 \cos\Omega_1 t_1 - F_y \cos\Omega_2 t_2 \sin\Omega_1 t_1 + F_x \sin\Omega_2 t_2 \sin\Omega_1 t_1$$

FID4

$$\xrightarrow{t_1} I_x \cos\Omega_1 t_1 + I_y \sin\Omega_1 t_1 \xrightarrow{TROP\ AE-mix} S_x \cos\Omega_1 t_1 - S_y \sin\Omega_1 t_1 \xrightarrow{t_2} S_x \cos\Omega_2 t_2 \cos\Omega_1 t_1 + S_y \sin\Omega_2 t_2 \cos\Omega_1 t_1 - S_y \cos\Omega_2 t_2 \sin\Omega_1 t_1 + S_x \sin\Omega_2 t_2 \sin\Omega_1 t_1 \xrightarrow{TROP\ AE-mix} F_x \cos\Omega_2 t_2 \cos\Omega_1 t_1 - F_y \sin\Omega_2 t_2 \cos\Omega_1 t_1 + F_y \cos\Omega_2 t_2 \sin\Omega_1 t_1 + F_x \sin\Omega_2 t_2 \sin\Omega_1 t_1$$

Reconstruction of cos and sin modulated data is performed according to the table below.
Sum and subtract for dimension t_2

Σ_1= (FID1+FID2) = $2F_x \cos \Omega_2 t_2 \cos \Omega_1 t_1 - 2F_x \sin \Omega_2 t_2 \sin \Omega_1 t_1$

Δ_1= (FID1-FID2) = $2F_y \sin \Omega_2 t_2 \cos \Omega_1 t_1 + 2F_y \cos \Omega_2 t_2 \sin \Omega_1 t_1$

Σ_2= (FID3+FID4) = $2F_x \cos \Omega_2 t_2 \cos \Omega_1 t_1 + 2F_x \sin \Omega_2 t_2 \sin \Omega_1 t_1$

Δ_2= (FID3-FID4) = $2F_y \sin \Omega_2 t_2 \cos \Omega_1 t_1 - 2F_y \cos \Omega_2 t_2 \sin \Omega_1 t_1$

Sum and subtract for dimension t_1

$\Sigma_3 = \Sigma_1 + \Sigma_2 = 4F_x \cos \Omega_2 t_2 \cos \Omega_1 t_1$

$\Delta_3 = \Sigma_1 - \Sigma_2 = -4F_x \sin \Omega_2 t_2 \sin \Omega_1 t_1$

$\Sigma_4 = \Delta_1 + \Delta_2 = 4F_y \sin \Omega_2 t_2 \cos \Omega_1 t_1$

$\Delta_4 = \Delta_1 - \Delta_2 = 4F_y \cos \Omega_2 t_2 \sin \Omega_1 t_1$

		In t_1	
		cos	sin
In t_2	cos	Σ_3	Δ_4
	sin	Σ_4	Δ_3

In conclusion, sensitivity enhancement is retained in both cases where mixed States and Echo-Antiecho approaches are used. States procedure with single component selection just prevents sensitivity enhancement in that particular time domain. Likewise, water suppression, which is connected with selection of a single component of magnetization, prevents sensitivity enhancement just in one time domain after which it is applied.

Setup of sensitivity-enhanced experiments

Parameter optimization

The setup of sensitivity enhanced experiments generally follows the same steps usually necessary for CP-based experiments. When using the provided pulse sequences (see below or in the data publication) all pulses are annotated in Topspin with respect to their function, and, where applicable, their shape, length and power level. Remaining CP elements can be chosen as preferred by the user, whereas TROP pulses have a defined power level and pulse length that are optimized, too. In our hands, the theoretical RF power always gives signal, which can be further optimized, e.g., by running popt. A range between the theoretical value (in dB) stepwise decreased down to 4 dB less than the maximum gives good coverage. Higher power levels can be chosen as well but will cause a higher load on the electronics and sample.

Table S1 summarizes the rf-fields used in the presented sensitivity-enhanced experiments.

RF parameters

Table S1: RF parameters of CPs and TROPs for all experiments for which sensitivty-enhancements are reported in the main text.

sehCACONH				
Hard pulses	µs	RF / kHz		
^{1}H	1.75	142.9		
^{15}N	4.95	50.5		
^{13}C	2.80	89.3		
H-C CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	1500	18.92		36.84
Shape		100-50 tang.		rectangular
homoTROP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	1782			60.92
Shape				CACO homoTROP
CO-N TROP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	3600		46.81	69.71
Shape			CON TROP N	CON TROP C
N-H TROP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	792	90.66	68.44	
Shape		NH TROP H	NH TROP N	
hCACONH				

Hard pulses	µs	RF / kHz		
^{1}H	1.75	142.9		
^{15}N	4.95	50.51		
^{13}C	2.80	89.29		

H-C CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	1500	18.92		38.57
Shape		100-50 tang.		rectangular

CACO BSH CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	8000			23.59
Shape				80-100 ramp

CO-N CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	11000		33.37	20.06
Shape			90-100 ramp	rectangular

N-H CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	150	12.85	43.48	
Shape		50-100 tang.	90-100 ramp	

sehCOCANH

Hard pulses	µs	RF / kHz		
^{1}H	1.75	142.9		
^{15}N	4.95	50.5		
^{13}C	2.80	89.3		

H-C CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	1500	18.92		36.84
Shape		100-50 tang.		rectangular

homoTROP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	1782			60.92
Shape				COCA homoTROP

CA-N TROP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	3600		46.81	71.33
Shape			CAN TROP N	CAN TROP C

N-H TROP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	792	99.98	70.04	
Shape		NH TROP H	NH TROP N	

hCOCANH

Hard pulses	µs	RF / kHz		
^{1}H	1.75	142.9		
^{15}N	4.95	50.5		
^{13}C	2.80	89.3		

H-C CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	1500	18.92		38.57
Shape		100-50 tang.		rectangular

COCA BSH CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	8000			23.59
Shape				80-100 ramp

CA-N CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	11000		33.37	20.06
Shape			90-100 ramp	rectangular

N-H CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	150	12.85	43.48	
Shape		50-100 tang.	90-100 ramp	

3-fold seHNcoCANH (800 MHz)

Hard pulses	µs	RF / kHz		
^{1}H	1.63	153.4		
^{15}N	5	50.0		
^{13}C	2.80	89.3		

H-N CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	800	40.02	22.44	
Shape		100-50 tang.	rectangular	

N-CO TROP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	3600		41.73	57.98
Shape			NCO TROP N	NCO TROP C

homoTROP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	1782			63.87
Shape				COCA homoTROP

CA-N TROP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	3600		45.76	62.13
Shape			CAN TROP N	CAN TROP C

N-H TROP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	792	81.24	68.39	
Shape		NH TROP H	NH TROP N	
HNcoCANH (800 MHz)				
Hard pulses	µs	RF / kHz		
^{1}H	1.63	153.4		
^{15}N	5	50.0		
^{13}C	2.80	89.3		
H-N CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	800	40.02	21.93	
Shape		100-50 tang.	rectangular	
N-CO CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	11000		33.03	21.49
Shape			90-100 ramp	rectangular
COCA BSH CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	6500			22.27
Shape				80-100 ramp
CA-N CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	11000		33.03	21.49
Shape			90-100 ramp	rectangular
N-H CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	150	40.02	21.93	
Shape		50-100 tang.	rectangular	
2-fold HNcoCANH (500 MHz)				
Hard pulses	µs	RF / kHz		
^{1}H	1.00	250.0		
^{15}N	3.9	64.1		
^{13}C	2.40	104.2		
H-N CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	150	48.35	16.71	
Shape		100-50 tang.	rectangular	
N-CO CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	11000		38.18	15.95
Shape			100-90 ramp	rectangular

COCA BSH CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	5000			24.70
Shape				100-90 ramp

CA-N TROP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	3600		49.02	71.32
Shape			CAN TROP N	CAN TROP C

N-H TROP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	792	79.42	62.36	
Shape		NH TROP H	NH TROP N	

HNcoCANH (500 MHz)

Hard pulses	µs	RF / kHz		
^{1}H	1.00	250.0		
^{15}N	3.9	64.1		
^{13}C	2.40	104.2		

H-N CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	150	48.35	16.14	
Shape		100-50 tang.	rectangular	

N-CO CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	11000		38.18	15.95
Shape			100-90 ramp	rectangular

COCA BSH CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	5000			24.99
Shape				100-90 ramp

CA-N CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	11000		38.18	15.06
Shape			100-90 ramp	rectangular

N-H CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	150	48.35	16.14	
Shape		50-100 tang.	rectangular	

sehcaCBCANH

Hard pulses	µs	RF / kHz
^{1}H	0.98	255.1
^{15}N	3.75	66.7
^{13}C	2.40	104.2

H-C CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	2000	50.32		13.72
Shape		100-50 tang.		rectangular
CACB INEPT		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
INEPT delay	13.2 ms			
CA-N TROP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	3600		44.71	66.56
Shape			CAN TROP N	CAN TROP C
N-H TROP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	792	67.25	65.30	
Shape		NH TROP H	NH TROP N	
		hcaCBCANH		
Hard pulses	µs	RF / kHz		
^{1}H	0.98	255.1		
^{15}N	3.75	66.7		
^{13}C	2.40	104.2		
H-C CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	2000	50.32		13.72
Shape		100-50 tang.		rectangular
CACB INEPT		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
INEPT delay	13.2 ms			
CA-N CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	11000		39.71	15.06
Shape			100-90 ramp	rectangular
N-H CP		RF ^{1}H / kHz	RF ^{15}N / kHz	RF ^{13}C / kHz
Contact time	150	50.32	14.70	
Shape		50-100 tang.	rectangular	

Acquisition

Acquisition parameters such as TD points, spectral widths and carriers can be set as usual.

To configure NUS, the FnType must be set to *non-uniform_sampling*. In the NUS tab the number of *NUSpoints* can be set, e.g., 2048. Spectra with many peaks, e.g., NOESY/RFDR or such of large proteins, generally require more NUS points for sufficient quality, whereas small proteins like the SH3-domain yield good spectra with fewer points. For simplicity one can choose the *Calculate* button in TopSpin to create a NUS schedule. More advances users might use the schedule generator of their choice!

To setup echo / antiecho in sensitivity-enhanced dimensions, the FnMODE must be set to *Echo-Antiecho* in the respective dimensions, while all other dimensions will be set to *States-TPPI*. Table S2 summarizes the setting for each presented spectrum.

Besides, no further considerations must be made!

We highly recommend to record 2D planes for each indirect dimension initially, e.g., by setting the TD points of all other indirect dimensions to zero. The FnType must then be set to *traditional!* This is to ensure that you observe the right correlations and to have initial values for phase correction.

Table S2: FnMode selection as required in TopSpin for the experiments presented in the main text.

	FnMode as set in Topspin (with description of dimension)				
Experiment	**F4**	**F3**	**F2**	**F1**	
	H	N	CO	Ca	
hCACONH	Direct dimension	Echo-Antiecho	Echo-Antiecho	Echo-Antiecho	
	H	N	CA	CO	
hCOCANH	Direct dimension	Echo-Antiecho	Echo-Antiecho	Echo-Antiecho	
	H	N	CA	CB	
2-fold hcaCBCANH	Direct dimension	Echo-Antiecho	Echo-Antiecho	States-TPPI	
	F5	**F4**	**F3**	**F2**	**F1**
	H	N	Ca	H_{i+1}	N_{i+1}
2-fold HNcoCANH	Direct dimension	Echo-Antiecho	Echo-Antiecho	States-TPPI	States-TPPI
	H	N	CA	N_{i+1}	H_{i+1}
3-fold HNcoCANH	Direct dimension	Echo-Antiecho	Echo-Antiecho	Echo-Antiecho	States-TPPI

NUS Processing of sensitivity-enhanced experiments

Overview

Table S3: Overview over the different processing workflow for the various sensitivity-enhanced experiments. Details for exemplary workflows can be found below.

	4D experiments			5D experiments
Reconstruction method	SSA	SMILE	hmsIST	SSA
Processing framework	SSA (none)	NMRPipe	NMRPipe	SSA (none)
Preferred type of input data for reconstruction	Varian	NMRPipe	NMRPipe	Varian
Converting input data	Run bruk2ssa in the respective directory from the terminal	1. Modify the provided `fid.com` scripts *or* run bruker and explicitly use the NUS option 2. Add rance_kay shuffling where applicable 3. Run `fid.com`	1. Modify the provided `fid.com` scripts *or* run bruker and explicitly use the NUS option 2. Add rance_kay shuffling where applicable 3. Run `fid.com`	Run bruk2ssa in the respective directory from the terminal
Before the reconstruction	Update / check parameters.txt with the following changes: 1. Phase correction (inverted sign from Topspin) 2. Carrier offsets (will be autom.) 3. Zero filling 4. Apodization	1. Phase correction. Use TopSpin values with inverted sign. 2. Ideally, perform a 1D FT for checking	3. Phase correction. Use TopSpin values with inverted sign. 4. Ideally, perform a 1D FT for checking	Update / check parameters.txt with the following changes: 1. Phase correction (inverted sign from Topspin) 2. Carrier offsets (will be autom.) 3. Zero filling 4. Apodization Prepare 3D source peak list[1,2]
Reconstruction	Run cleaner4d and afterwards reconstructor 4d	Run smile.com	Run hmsIST.com	Run cleaner5D and afterwards reconstructor5D
After reconstruction	No further steps; SSA already did the FT for you	No further steps if smile.com is used. FT is done automatically. Can be stopped after reconstruction to perform FT stepwise	No further steps if smile.com is used. FT is done automatically. Can be stopped after reconstruction to perform FT stepwise	No further steps; SSA already did the FT for you

Processing workflows

In the following section you find commented step-by-step instructions for the processing workflows of the different experiments depending on their processing environment. We recommend following these once with the data provided in our data publication (https://data.tu-dortmund.de/previewurl.xhtml?token=2bbfe189-be14-4fa9-8b4f-b25aefc89e82) before modifying parameters.

Processing with SSA using bruk2ssa

When using SSA for signal reconstruction it is easiest to use bruk2ssa (provided with the data publication and available as download at https://data.tu-dortmund.de/previewurl.xhtml?token=2bbfe189-be14-4fa9-8b4f-b25aefc89e82) as setup script. bruk2ssa will automatically reads acquisition and selected processing parameters, e.g., for exponential line broadening, directly from Topspin, and convert Bruker data to Varian data for compatibility reasons.

Installation of SSA and bruk2ssa under Linux (tested for Ubuntu 24):

1. Download bruk2ssa from the data publication

2. Download the SSA (64 bit) software package from http://nmr.cent3.uw.edu.pl/software/category/4-ssa-program-for-removing-of-nus-artefacts-3d-4d-program-for-reconstruction-of-full-4d-spectra-or-computing-2d-cross-sections-qsparseq-ft-from-4d-data-obtained-by-4d-ssa-above and unpack the zip file

3. Download the MFT (64 bit) package from http://nmr.cent3.uw.edu.pl/software/category/1-suite-of-applications-for-mft-and-qsparseq-ft-toastd-reduced-cubes-timetabgen

4. Save bruk2saa and SSA in a dedicated folder and from the MFT folder only copy the file toastd into your SSA folder.

5. Add the following lines to your `.bashrc` file located in your home directory by using any text editor of your choice. If you don't see the file, please check if hidden files are shown. You can do so usually by pressing Ctrl + H.

   ```
   export PATH=$PATH:/path/to/your/folder/bruk2ssa/bin (e.g. export PATH=$PATH/home/alexander/SSA/bruk2ssa/bin

   export PATH=$PATH:/path/to/your/folder/SSA (e.g. export PATH=$PATH/home/alexander/SSA)
   ```

6. Log out and in again or restart your computer

Reconstruction with SSA

To prepare the reconstruction, some required files for SSA have to be created which is done by bruk2ssa.

1. Move to the directory containing your sparse data set either by using the terminal or the file explorer. In the latter case right click on an empty field in your data set and choose “open in terminal”.

2. Execute bruk2ssa simply by typing `bruk2ssa` in the terminal and pressing Enter. The script will generate some output that should be checked for error messages and further information. If successful, the following files will be created
   - schedule.txt (based on nuslist but as fraction of tmax rather than TD points)
   - varian.fid (the sparse FID in Varian format)
   - parameters.txt (containing acquisition and processing parameters for the reconstruction)

   An easy way to check for correct conversion is to compare the size of the newly created varian.fid file and the ser file. Varian.fid should be slightly larger compared to the ser file but approximately have the same size.

3. Open the file `parameters.txt` When using bruk2ssa with unmodified versions of the provided pulse programs, it will automatically adjust the carrier when jumps occur during the experiments, e.g., in an hCACONH with a dedicated Ca and a dedicated CO axis. Nevertheless, the parameter file should be checked for correctness.

   Below is an example for the fully enhanced hCACONH experiment.

```
input_file ./varian.fid
ni 1024                 # NUS points; should match nuslist
fcoef 1 0 0 0 0 0 -1 0
bioref 1
fixed 2 0 0 1
VD_mode 0
J_COCA 53.7

name 1H
np 1024                 # Points used from the direct dim
fnz 2048                # Zero filling direct dimension
sw 20833.3333333333
sf 800.273761269        # Carrier frequency in MHz
reffrq 800.27           # Larmor freq. For respective nucleus
ssfilter 0 0
fp_mult 1
phases 47.2 -12240.0    # 0th and 1st order phase correction
weight_lb 1 50          # Line broadening as in Topspin
weight_sb 0 -0.04915200 # similar to QSINE / SINE; adjusted automatically
weight_sbs 0 -0.0491520ß# similar to QSINE / SINE; adjusted automatically
weight_gf 0 0
weight_gfs 0 0
weight_awc 0 0

name1 13C
sw1 9259.25925925926
phases1 -90 0           # 0th and 1st order phase correction
sf1 201.23911954286     # Carrier in MHz (adjusted autom.); here Ca
reffrq1 201.228052      # Larmor freq. For respective nucleus
tmax1 0.011988          # acquisition time along dimension
fnz1 336.0              # Zero filling
weight_lb1 0 0          # window functions; LB and QSINE automatically
```

```
weight_sb1 0 0
weight_sbs1 0 0
weight_gf1 0 0
weight_gfs1 0 0

name2 13C
sw2 3968.25396825397
phases2 0 0             # 0th and 1st order phase correction
sf2 201.23911954286     # Carrier in MHz (adjusted autom.); here CO
reffrq2 201.228052      # Larmor freq. For respective nucleus
tmax2 0.029987999999999987 # acquisition time along dimension
fnz2 368.0              # Zero filling
weight_lb2 0 0          # window functions; LB and QSINE automatically
weight_sb2 0 0
weight_sbs2 0 0
weight_gf2 0 0
weight_gfs2 0 0

name3 15N
sw3 3267.97385620915
phases3 0 0             # 0th and 1st order phase correction
sf3 81.10038624         # Carrier in MHz (adjusted autom.)
reffrq3 81.090655       # Larmor freq. For respective nucleus
tmax3 0.039168          # acquisition time along dimension
fnz3 384.0              # Zero filling
weight_lb3 0 0          # window functions; LB and QSINE automatically
weight_sb3 0 0
weight_sbs3 0 0
weight_gf3 0 0
weight_gfs3 0 0
```

For more details on the parameter file please see documentation for MFT downloadable under http://nmr.cent3.uw.edu.pl/software/category/1-suite-of-applications-for-mft-and-qsparseq-ft-toastd-reduced-cubes-timetabgen

4. You can adjust zero filling and apodization to your taste, but the values set in TopSpin should appear correctly here for selected processing parameters.

5. To perform the reconstruction with SSA, you have to run two programs, called cleaner and afterwards reconstructor with certain options. When simply running cleaner3d[3], cleaner4d[4] or cleaner5d[1] without any parameters you will see a full list of adjustable parameters. To do so, simply type the respective command into your terminal and press Enter. Details on the functionality of each version can be found in the literature. For 5D processing we strongly suggest checking the literature[1,2] for a detailed description on the functionality and workflow as an additional 3D (se)hCANH experiment is required! Below we suggest a default that can simply be copied to the terminal for the respective data set

   sehCACONH: `cleaner4d -vv -j 16 --roi=5:10 --flip=101 schedule.txt`
   sehCOCANH: `cleaner4d -vv -j 16 --roi=5:10 --flip=101 schedule.txt`
   2-fold sehcaCBCANH: `cleaner4d -vv -j 16 --roi=5:11 --flip=101 schedule.txt`

2-fold seHNcoCANH: `cleaner5d -vv -j 16 --roi=5:10 --flip=1000 -p hCANH.list -F 30012 -o 1000_clean schedule.txt`
3-fold seHNcoCANH: `cleaner5d -vv -j 16 --roi=6:13 --flip=1000 -p hCANH_assigned.list -F 30012 -o 1000 schedule.txt`

If you intend to perform Fourier transformation of the sparse data set (nuFT) simply insert the option `--maxiter=0` anywhere before `schedule.txt`

Please note: The `-j` parameter should be adjusted to the hardware configuration of your computer. If you are unsure, please use 4! The `roi` parameter sets the region of the direct dimension that is considered. Adjust to your taste and needs.

6. When the cleaner has run successfully, simply run the reconstructor as suggested below. If you used a different range for the direct dimension (roi), the numbers, here 5 and 10, will change and you have to use yours.
sehCACONH: `reconstructor4d -vv -j 16 --full roi_5_10`
sehCOCANH: `reconstructor4d -vv -j 16 --full roi_5_10`
2-fold sehcaCBCANH: `reconstructor4d -vv -j 16 --full roi_5_11`
2-fold seHNcoCANH: `reconstructor5d -vv -j 16 -p hCANH.list -F 30012 --proj 1000_clean/`
3-fold seHNcoCANH: `reconstructor5d -vv -j 16 -p hCANH_assigned.list -F 30012 --proj 1000/`

   Please note: The `-j` parameter should be adjusted to the hardware configuration of your computer. If you are unsure, please use 4! The `full` parameter can be changed to `--proj` to only get projections. This will save disk space if you want to have a first look at your spectra.

   This will create a Fourier-transformed spectrum in the roi_A_B folder created by the cleaner program, which can directly be loaded into common NMR software packages for further work.

7. Congratulations! You completed your reconstruction with SSA. To compare your results you can use the projections of the spectrum provided in the roi folders of the SSA directory.

Every time you change a processing parameter, you must run cleaner and reconstructor again! Also, be aware that using a window function in the indirect dimensions will result in a mixed apodization if a weighted NUS schedule was used.

Processing with SMILE and NMRPipe

Installation of SMILE and NMRPipe: SMILE[5] uses NMRPipe[6] for data conversion and essentially all operations that are not directly related to the reconstruction itself. It is therefore necessary to have NMRPipe installed. *If you would like to avoid any local installations, we highly recommend the NMRbox[7] platform, which has all required software packages installed!*

It should be noted that for both, SMILE and hmsIST, we follow the NUSPipe workflow as it is well documented on the NMRPipe webpage and closely resembles the processing of uniform-sampled data sets. However, it might be slower and more resource intensive.

If you use NMRbox you can skip this step.

1. Download and install NMRPipe by following the steps described in https://www.ibbr.umd.edu/nmrpipe/install.html

   Include the SMILE plugin and it will automatically be installed and available afterward.

Data conversion with NMRPipe:

1. Move to the directory containing your sparse data set either by using the terminal or the file explorer. In the latter case right click on an empty field in your data set and choose “open in terminal”.

2. When using the demo data, move to the SMILE subdirectory. It contains two executables, named `fid.com` and `smile.com`. Former performs data conversion including filling all skipped data points in the multidimensional FID with zeros. The latter one will reconstruct and Fourier-transform the data set. The fid.com can also be obtained by starting the bruker program contained in NMRpipe, simply by entering bruker in the terminal and starting it. Below you can find the commented script used to process the sehCOCANH data set as well as the hCOCANH to point out the essential differences. For processing, please use the uncommented version provided in the data publication!

Caution! The data sets provided as demo are high-resolution data sets and therefore will (temporarily) occupy plenty of disk space. Make sure you have sufficient, i.e., > 400 GB of disk space available

```
#!/bin/csh

# This fills in all missing data points with zeros, creating ser_full
nusExpand.tcl -mode bruker -sampleCount 1024 -avg -off 0 \
 -in ./ser -out ./ser_full -sample ./nuslist

#This is the actual conversion from the Bruker data (ser) to NMRPipe
bruk2pipe -verb -in ./ser_full \
  -bad 0.0 -ext -aswap -AMX -decim 960 -dspfvs 20 -grpdly 68  \

# Those are the total points in every dimension
  -xN              2048  -yN               256  -zN               238  -aN               238  \

# Those are the time points in every dimension (usually half of above)
  -xT              1024  -yT               128  -zT               119  -aT               119  \

# Acquisition type, similar to FnMode. For Echo-Antiecho this must be Complex!
  -xMODE            DQD  -yMODE  Complex  -zMODE  Complex  -aMODE  Complex  \

# Spectral widths as used in Topspin
  -xSW        20833.333  -ySW         3267.974  -zSW         9259.259  -aSW         3968.254  \

# Larmor frequencies of the respective nuclei
  -xOBS         800.274  -yOBS          81.100  -zOBS         201.263  -aOBS         201.263  \

# Carriers for each dimension in ppm
  -xCAR           4.700  -yCAR         120.004  -zCAR         55.000  -aCAR         173.000  \
```

```
# Axis label
  -xLAB              HN  -yLAB             15N  -zLAB            13CA  -aLAB            13CO  \

# Number of total dimensions. -aq2D must be Complex here as well
  -ndim               4  -aq2D         Complex                          \

# These lines convert each Echo-Antiecho dimension independently to NMRPipe default. If, e.g,
your A dimension is not Echo-Antiecho the third line will be deleted
| nmrPipe -fn MAC -macro $NMRTXT/bruk_ranceY.M -noRd -noWr \
| nmrPipe -fn MAC -macro $NMRTXT/bruk_ranceZ.M -noRd -noWr \
| nmrPipe -fn MAC -macro $NMRTXT/bruk_ranceA.M -noRd -noWr \

# Write NMRpipe files
| pipe2xyz -x -out ./fid/test%04d.fid -ov

# Create necessary files needed for NUS reconstruction
xyz2pipe -in ./fid/test%04d.fid -noWr \
| nusExpand.tcl -mask -noexpand -mode pipe -sampleCount 1024 -avg -off 0 \
  -in stdin -out ./mask/test%04d.fid -sample ./nuslist
```

3. To run the fid.com file, copy into the next higher directory, i.e., the one containing the ser file as well as the acqus files, etc. Then simply run ./fid.com in the terminal. Make sure the file can be executed by typing chmod u+x fid.com. Be patient.

4. After conversion, the direct dimension will be processed, followed by reconstruction with SMILE and Fourier-transformation of the indirect dimensions. The smile.com script in the demo data will do all of this in one step, but below you can see a commented version, highlighting the individual steps. If you want to reproduce only some steps, e.g,, becaue you want to change the processing parameters along a given dimension, you can either comment the undesired commands out but placing a # in front or copy the desired lines into a new file and run this one.

   Note that for the changes to be reflected in the spectrum, *all* consecutive steps must be repeated, but not the preceding ones. Make sure that phasing and processing of the direct dimension is correct, as the subsequent reconstruction is the most time-consuming step.

Caution! SMILE automatically determines the resources, e.g., number of CPUs and memory, available on your computer. Nevertheless, significant amount of memory might be required. The more points, the more memory and disk space you will need!

```
#!/bin/csh

#
# Generated by basicFT4.com Version 2023.165.15.00
#
# basicFT4.com -progName smile4D.com -nus -smile -sample nuslist \
#              -in fid/test%04d.fid -sample nuslist -out smile_nonuszf/test%03d.ft4 \
#              -xEXTX1 12ppm -xEXTXN 5ppm -nonusZF -yNUSZF zf=0 -zNUSZF zf=0 \
#              -aNUSZF zf=0 -yFTARG alt -zFTARG alt -aFTARG alt -xP0 -103 \
#              -smileArgs nThread=12 -ssNMR
#


#
# Arguments duplicated later on the SMILE command line are ignored:

# This imports the previously generated FID in NMRPipe format
xyz2pipe -in fid/test%04d.fid -x -verb \
| nmrPipe -fn EM -lb 50 \ # Apply 50 Hz of line broadening
| nmrPipe -fn FT \ # Fourier transformation
| nmrPipe -fn PS -p0 35.0 -p1 0.0 -di \ # Phase correction
| nmrPipe -fn EXT -x1 12ppm -xn 5ppm -sw \ # Truncate the direct dimension to the signal region
of interest
```

```
| pipe2xyz -out tmp/test%05d.ft1 -a -ov # Write the data along the a-axis first

xyz2pipe -in tmp/test%05d.ft1 -x \ # Read the data along the x-axis
# Start SMILE. Only indirect dimensions are considered, so y becomes x, z becomes y, and a
becomes z.
| nusPipe -fn SMILE -nocheckArgs -nDim 4 -maxIter 2048 -report 1 -sample nuslist \
                    -xT 128 -xP0 90.0 -xP1 0.0 \ # Processing parameters along x-Axis
                    -yT 119 -yP0 90.0 -yP1 0.0 \ # Processing parameters along y-Axis
                    -zT 119 -zP0 0.0 -zP1 0.0 \ # Processing parameters along z-Axis
                    -xNeg -zNeg \ # The first and last dimension have to inverse FT (depends on
              the data set and is not always the case!)
# Write the reconstructed data
| pipe2xyz -out ft1/test%05d.ft1 -x -ov
```

5. At this point your spectrum is reconstructed. The following steps now perform stepwise Fourier-transformations of the indirect dimensions.

```
# FT of first (F3) indirect dimension
xyz2pipe -in ft1/test%05d.ft1 -x -verb \
| nmrPipe -fn ZF -size 256 \ #Zero filing
| nmrPipe -fn FT -neg \ # FT with flip
| nmrPipe -fn PS -p0 90.0 -p1 0.0 -di \ # Phase correction
| nmrPipe -fn TP \ # Transpose the data hypercube
| nmrPipe -fn ZTP \ # Z-transpose the hypercube
| pipe2xyz -out ft2/test%05d.ft2 -a -ov # Write the data

# FT of second (F2) indirect dimension
xyz2pipe -in ft2/test%05d.ft2 -z -verb \
| nmrPipe -fn ZTP \
| nmrPipe -fn ZF -size 238 \
| nmrPipe -fn FT \
| nmrPipe -fn PS -p0 90.0 -p1 0.0 -di \
| nmrPipe -fn ZTP \
| pipe2xyz -out ft3/test%05d.ft3 -x -ov

# FT of third (F1) indirect dimension
xyz2pipe -in ft3/test%05d.ft3 -a -verb \
| nmrPipe -fn ZF -size 238 \
| nmrPipe -fn FT -neg \
| nmrPipe -fn PS -p0 0.0 -p1 0.0 -di \
| pipe2xyz -out smile_nonuszf/test%03d.ft4 -a -ov

# Calculate projections along every dimension
proj4D.tcl -in smile_nonuszf/test%03d.ft4 -axis -outDir nusproj

# Remove temporary files !! Comment this line if you want to keep the data to optimize processing
!!
/bin/rm -rf ft3 ft1 ft2 tmp

# Combine into a single spectrum (can then be loaded in to Poky, CCPNmr, etc.)
xyz2pipe -in smile_nonuszf/test%03d.ft4 > sehCOCANH.ft

# Convert to UCSF (optional; requires POKY)
#pipe2ucsf sehCOCANH.ft sehCOCANH.ucsf
```

6. Congratulations! Your SMILE reconstruction is complete. To compare your results you can use the projections of the spectrum provided in the nusproj folder of the SMILE directory.

Processing with hmsIST and NMRPipe

Installation of hmsIST and NMRPipe: hmsIST[8] also uses NMRPipe[6] for data conversion and all other operations. Therefore, please follow the setup instructions given in the SMILE instructions above. As before for SMILE the below example describes the reconstruction of the sehCOCANH data set.

1. Note, that for this workflow your hmsIST version **must** support the padded NMRpipe format in addition to the phf format. *The version available on NMRbox does so and is therefore recommended!*

Data conversion with NMRPipe:

2. This step is the same as shown for the SMILE workflow and the fid.com file in the hmsIST subdirectory is indeed the same as for SMILE. *Please read the above section for details*! Again (for later uses) keep in mind that the Echo-Antiecho conversion is only necessary for sensitivity-enhanced dimensions. Here, those are all three, but in case of the 2-fold enhanced hcaCBCANH the lines would look this this

```
# These lines convert each Echo-Antiecho dimension independently to NMRPipe default.
| nmrPipe -fn MAC -macro $NMRTXT/bruk_ranceY.M -noRd -noWr \
| nmrPipe -fn MAC -macro $NMRTXT/bruk_ranceZ.M -noRd -noWr \
# The last, a-dimension in the sehcaCBCANH experiment is States-TPPI and requires no conversion.
```

3. As hmsIST also uses the NUSPipe workflow the steps closely follow those for SMILE processing and reverse. Essentially, only the actual reconstruction step differs. For completion, the hmsist.com script in its commented version is described below. To run it, please copy the hmsist.com and box_parallel files from the hmsIST directory into the folder containing the Bruker ser and acqu files.
   hmsIST by itself does not support parallel computing but is highly suited for this. An effective workaround is to launch multiple instances of hmsIST as each one processes only one data point along the direct dimension at a time. This is done by using GNUparallel[9] which is available on NMRbox without further steps.

Caution! Large amounts of data will be loaded into the memory. As mentioned before, this strongly depends on the size of your spectra. The herein presented demo data will use around 25 GB of memory per CPU! The number of utilized CPUs by parallel can be adjusted through the -j parameter in the respective line.

```
#!/bin/csh

#
# Generated by basicFT4.com Version 2020.055.12.15
#
# basicFT4.com -progName hmsIST4D.com -nus -hmsIST \
#              -in fid/test%04d.fid
#

# Remove data from previous runs and recreate the output directory
rm -rf ft1_ist
mkdir ft1_ist

# Read the converted FID
xyz2pipe -in fid/test%04d.fid -x -verb \
| nmrPipe -fn EM -lb 50 \ # Apply 50 Hz of line broadening
| nmrPipe -fn FT \ # Fourier transformation
```

```
| nmrPipe -fn PS -p0 35.0 -p1 0.0 -di \ # Phase correction
| nmrPipe -fn EXT -x1 11ppm -xn 5ppm -sw \ # Restrict direct dimension to region of interest
| pipe2xyz -out tmp/test%05d.ft1 -a -ov # Write the data

# Here we call the script box_parallel, which in turn starts hmsIST (see below). Using
GNUparallel this runs in parallel to speed up reconstruction.

parallel -j 20 ./box_parallel ::: tmp/*

# The reconstruction is now complete. The steps below perform FT along the indirect dimensions.
# Read reconstructed FID
xyz2pipe -in ft1_ist/test%05d.ft1 -x -verb \
#| nmrPipe -fn ZF -size 64 \ # Zero filling
| nmrPipe -fn FT -neg\ # FT and flip spectrum by negating imaginary components
| nmrPipe -fn PS -p0 90.0 -p1 0.0 -di \ # Phase correction
| nmrPipe -fn TP \ # Transpose the hypercube
| nmrPipe -fn ZTP \ # Transpose along Z
| pipe2xyz -out ft2/test%05d.ft2 -a -ov # Write data

# Read data back in along z
xyz2pipe -in ft2/test%05d.ft2 -z -verb \
| nmrPipe -fn ZTP \ # Perform z-transpose
#| nmrPipe -fn ZF -size 64 \ # Zero filling
| nmrPipe -fn FT \ # Fourier transformation
| nmrPipe -fn PS -p0 90.0 -p1 0.0 -di \ # Phase correction
| nmrPipe -fn ZTP \ # Z-transpose
| pipe2xyz -out ft3/test%05d.ft3 -x -ov # Write data now with 2/3 ind. Dimension processed

# Read data back along remaining dimension
xyz2pipe -in ft3/test%05d.ft3 -a -verb \
#| nmrPipe -fn ZF -size 64 \ # Zero filling
| nmrPipe -fn FT -neg \ # FT and flip spectrum by negating imaginary components
| nmrPipe -fn PS -p0 0.0 -p1 0.0 -di \ # Phase correction
| pipe2xyz -out hms_ist/test%04d.ft4 -a -ov # Write final data

# Compute projections along every axis
proj4D.tcl -in hms_ist/test%04d.ft4 -axis -outDir nusproj

# Remove temporary files !! Comment this line if you want to keep the data to optimize processing
!!
/bin/rm -rf ft3 ft1 ft2 tmp
```

4. GNUparallel will call the script `box_parallel` which is described below. The demo data contains a box_parallel script for each provided data set. The script essentially reads in the individual data points and feeds them each to one of the processes running in parallel. Important parameters are explained in the comments. hmsIST supports an automatic abortion function by setting the -itr parameters to -1. Generally, more iterations tend to make the spectrum better, while too many will cause attempts to reconstruct noise. Naturally, more iterations will take more time and as hmsIST is already the most resource intensive method presented here, -itr was set to 3, to provide a relatively fast reconstruction.

```
#!/bin/csh

# Check for input argument
if ($#argv != 1) then
    echo "Usage: $0 inputFile"
    exit 1
endif

# Extract input filename and output filename
set fullPath = $argv[1]
set inFile   = $fullPath:t
set outFile  = $inFile:r.ft1

# Run hmsIST on this file
# Padded pipe to support the provided data format. Otherwise phf is assumed
```

```
# -dim is the number of indirect dimensions
# -x,y,zN defines to total number of ouput points
# -itr defines the number of iterations. 3 are chosen here to speed up reconstruction. If set to
-1, hmsIST will automatically determine this

hmsIST -format padded_pipe -dim 3 -incr 1 -xN 256 -yN 238 -zN 238 \
    -itr 3 -verb 1 -sched ./nuslist \
    < $fullPath >! ./ft1_ist/$outFile
```

5. Congratulations. NUS reconstruction with hmsIST complete! To compare your results you can use the projections of the spectrum provided in the nusproj folder of the hmsIST directory.

Pulse programs in TopSpin / Bruker format

Triply enhanced 5D HNcoCANH

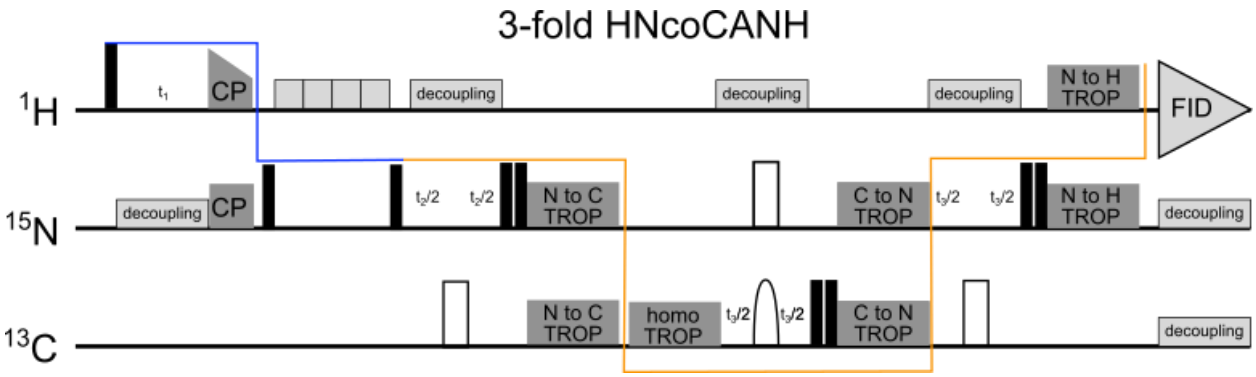

Fig. S4: Schematic representation of magnetization transfer steps in the triply enhanced HNcoCANH experiment. Blue represents normal transfers that do not yet carry both, x and y, components, while the orange line indicates transfers and dimensions which are sensitivity enhanced.

```
; seHNcoCANH.alkl_20250026v2
; 3-fold sensitivity-enhanced experiment using TROP pulses
; for NCO, COCA, CAN, NH transfers

; for references see:
; 1. Blahut, J., et al. (2022). JACS, 144(38): 17336-17340.
; 2. Blahut, J., et al. (2023). JMR Open: 100122.
; 3. https://optimal-nmr.net/


; transfer is H(F1)->N(F2)->CO->CA(F3)->N(F4)->H(F5)
; Assignment is in opposite direction

; f2 channel is Nitrogen
; f3 channel is Carbon

;=======================
; Variables introduction
;=======================
```

```
;p1   H p90 hard pulse
;p2   N p90 hard pulse
;p3   C p90 hard pulse
;p5   N p180 hard pulse
;p6   C p180 hard pulse
;p13  C selective p180 duration
;p14 soft 180 carbon on res. (sqrt(3)/(2*delta w))
;p15 HN CP contact time
;p17 CAN TROP pulse length (3600us @ 55.555 kHz)
;p19 NCO TROP length (3600 us @55.555 kHz)
;p21 NH TROP length (792us @ 55.555 kHz)
;p22 water suppression pulse length (10-25 ms)
;p27 homoTROP length (1782us @ 55.555 kHz)

;pl1  H p90 power
;pl2  N p90 power
;pl3  C hard power level
;pl5  N- NH CP power
;pl12 H decoupling power
;pl13 C selective 180 (Q3) power
;pl14 soft rectangular power (pi/2 equivalent)
;pl16 water suppression pulse power (10-20 kHz)
;pl22 decoupling on N (10-25 kHz)
;pl23 decoupling on C (10-25 kHz)
;pl27 N NCO CP power
;pl28 C NCO CP power

;sp2 NH H TROP power (100 kHz)
;sp3 NCO CO TROP power (80 kHz)
;sp7 CAN CA TROP power (80 kHz)
;sp8 H- HN CP power
;sp20 NH N TROP power (70 kHz)
;sp27 CAN N TROP power (55 kHz)
;sp12 C selective 180 power
;sp23 NCO N TROP power (55 kHz)
;sp28 homoTROP length (75 kHz)


;spnam2 TROP-NH-MAS55kHz44rp-chan1H.shp
;spnam3 TROP-NCO-MAS55kHz-200rp-chan13C.shp
;spnam7 TROP-CAN-MAS55kHz200rp-chan13C.shp
;spnam8 Tang75
;spnam20 TROP-NH-MAS55kHz44rp-chan15N.shp
;spnam23 TROP-NCO-MAS55kHz-200rp-chan15N.shp
;spnam27 TROP-CAN-MAS55kHz200rp-chan15N.shp
;spnam28 TROP-COCA-XXXMHz-MAS55kHz-99rp-chan13C.shp


;cpdprg1 H decoupling (low power)
;cpdprg2 WALTZ or similar
;cpdprg3 WALTZ or similar

;cnst19 offset for water suppression !!in Hz!!
;cnst10 offset for HN CP !!in Hz!!
;cnst31 CO offset in ppm (173 ppm)
;cnst30 carbon decoupling offset in ppm (110 ppm)
;cnst32 CA offset in ppm (55 ppm)

;============================
; Include file for Protection
;============================

#include <Avance.incl>
```

```
#include <Delay.incl>

;==============
; Set variables
;==============

;"cnst63 = plw12"
;"cnst62 = plw22"
;"cnst61 = plw23"
;"cnst60 = plw16"

"p6 = 2.0*p3"
"p5 = 2.0*p2"

"d11=30m"
"d21=0.2u"
"d10=0.2u"
"d20=0.2u"
"d30=0.2u"
; Fix TROP pulse length for 55.555 kHz
"p17=200*18u"
"p21=44*18u"
"p27=99*18u"

"in10=inf4/2" ;Ni dimension
"in30=inf3/2" ;CAi dimension
"in21=inf2/2" ;Ni+1 dimension
"in20=inf1/2" ;Hi+1 dimenson

"spoffs12 = (cnst31-cnst32)*bf3/1000000"
;============================
; Protection for parameters
;============================
1m
   if "p1 > 100u"     goto Problem
   if "p2 > 100u"     goto Problem
   if "p3 > 100u"     goto Problem
   if "p28 >10u"     goto Problem
  ; if "p28 >1000u"    goto Problem
   if "p15> 3000u"    goto Problem
   if "p16> 2000u"    goto Problem
   if "p17> 11001u"    goto Problem
   if "p18> 11001u"    goto Problem
   if "p29> 7000u"    goto Problem
   if "p22 >20001u"   goto Problem
   if "aq > 55m"      goto Problem
   if "d1 < 0.5s"     goto Problem
;   if "cnst63>0.2"     goto Problem
;   if "cnst62>1.1"     goto Problem
;   if "cnst61>0.1"     goto Problem
;   if "cnst60>0.2"     goto Problem

   goto PassParams
Problem, 1m
   print "Protection: Parameters not accepted, ending."
   goto HaltAcqu
PassParams, 1m


;----- Relaxation & reset parameters -----

1 ze
```

```
2 d11
  40u do:f3
  40u do:f2
  40u do:f1     ;decouplers off

3 d1
  20u reset:f1     reset:f2  reset:f3
  20u fq=cnst10:f1 fq=cnst31(bf ppm):f3 ; set offset to CO
  5u  pl1:f1       pl2:f2    pl3:f3

;----- 90 on H -----

  (p1 pl1 ph1):f1

;----- H (t1) evolution & N decoupling  -----
 1u pl22:f2 pl23:f3
 1u cpd2:f2 cpd3:f3
 d20*2.0
 2u do:f2 do:f3

;----- H/N CP -----

 (p15 pl5 ph13):f2  (p15:sp8 ph14):f1

;----- Water suppression  -----
;----- N magnetization along Z  -----

  (p2 pl2 ph17):f2


20u fq=cnst19:f1
2u pl16:f1
     p22:f1 ph22
     p22:f1 ph23
     p22:f1 ph22
     p22:f1 ph23

 20u fq=0:f1
 20u fq=cnst10:f1

;----- N magnetization in XY plane  -----

 (p2 pl2 ph18):f2

;----- N (t2) evolution & C decoupling  -----

  2u  pl12:f1  pl3:f3
  2u  fq=cnst30(bf ppm):f3
  1u  cpd1:f1
  d21
 (p3 ph20):f3
 (p6 ph21):f3
 (p3 ph20):f3
  d21
  2u fq=cnst31(bf ppm):f3
  2u do:f1
  1u
;----- N/CO CP -----

; echo-antiecho determination for N
(p2 pl2 ph5):f2           ; 1st half of 180
(p2 pl2 ph15):f2          ; 2nd half of 180
```

```
(p19:sp3 ph11):f3 (p19:sp23 ph10):f2


;----- CO to CA transfer  -----

(p27:sp28 ph29):f3
2u fq=cnst32(bf ppm):f3

;----- CA (t4) evolution & HC decoupling  -----
2u pl12:f1 cpd1:f1
  1u
  d30
  (center (p13:sp12 ph20):f3 (p5 pl2 ph20):f2)
  d30
 1u
2u do:f1
;----- Bloch - Siegert - shift compensation

(p14 pl14 ph20):f3
1u
 (center (p13:sp12 ph20):f3 (p5 pl2 ph20):f2)

;----- CA/N CP -----


; echo-antiecho determination for Ca
(p3 pl3 ph7):f3           ; 1st half of 180
(p3 pl3 ph8):f3           ; 2nd half of 180

; CaN seCP

(p17:sp7 ph2):f3 (p17:sp27 ph16):f2 ;(p32 pl51 ph13):f1
;----- N (t3) evolution & C decoupling  -----

  2u  pl12:f1  pl3:f3
  2u  fq=cnst31(bf ppm):f3
  2u  cpd1:f1
  d10
 (p3 ph20):f3
 (p6 ph21):f3
 (p3 ph20):f3
  d10
  6u do:f1

;----- N/H CP -----

  ; echo-antiecho determination
(p2 pl2 ph3):f2           ; 1st half of 180
(p2 pl2 ph4):f2           ; 2nd half of 180

; NH seCP
2u fq=cnst10:f1 fq=cnst32(bf ppm):f3
(p21:sp20 ph12):f2 (p21:sp2 ph10):f1

 2u pl22:f2 pl23:f3
 2u cpd2:f2  cpd3:f3
 2u fq=0:f1

 go=2 ph31
 d11 do:f2 do:f3  mc #0 to 2


 F1PH(calph(ph1, +90.0), caldel(d20, +in20)) ;1H i+1
```

```
 F2EA(calph(ph15, +180.0), caldel(d21, +in21)) ;15N i+1
 F3EA(calph(ph8, +180.0) & calph(ph6, +180), caldel(d30, +in30)) ;13CA i
 F4EA(calph(ph4, +180.0) & calph(ph8, +180.0), caldel(d10, +in10)) ;15N i


HaltAcqu, 1m
exit

;----- Phase cycling -----

ph1=  3 ; 1H excitation
ph2=  0

ph3 = 1
ph4 = 3

ph5 = 1
ph15 = 3
ph6 = 3

ph7 = 1
ph8 = 3

ph10= 0
ph11= 0 2
ph12= 0 0 0 0  2 2 2 2

ph13 = 0
ph14 = 0
ph16 = 0 0 2 2
ph17= 1
ph18= 3

ph20= 1
ph21= 2
ph22= 0
ph23= 1
ph29= 0
ph28 =1
ph31= 0 2 2 0  2 0 0 2

;=====================
; END Pulse program
;=====================
;; IMPORTANT NOTE
; On AV (avance) machines, its better to use "spf0" files instead of "sp0"
; The "sp0" files requires lot of time for power settings compared to "spf0"
```

Doubly enhanced 5D HNcoCANH

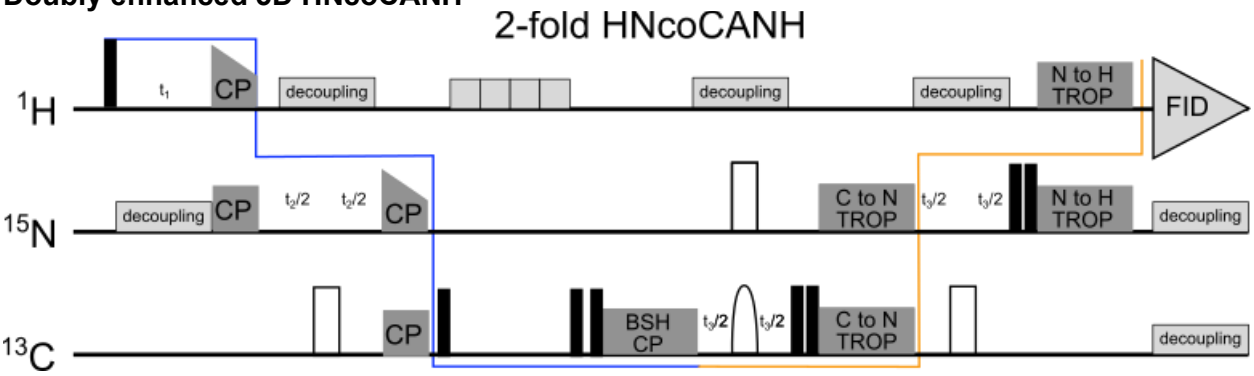

Fig. S5: Schematic representation of magnetization transfer steps in the doubly enhanced HNcoCANH experiment. Blue represents normal transfers that do not yet carry both, x and y, components, while the orange line indicates transfers and dimensions which are sensitivity enhanced.

```
; seHNcoCANH.alkl_20250026v2
; 2-fold sensitivity-enhanced experiment using TROP pulses
; for CAN, and NH transfers

; for references see:
; 1. Blahut, J., et al. (2022). JACS, 144(38): 17336-17340.
; 2. Blahut, J., et al. (2023). JMR Open: 100122.
; 3. https://optimal-nmr.net/


; transfer is H(F1)->N(F2)->CO->CA(F3)->N(F4)->H(F5)
; Assignment is in opposite direction

; f2 channel is Nitrogen
; f3 channel is Carbon

;=======================
; Variables introduction
;=======================

;p1   H p90 hard pulse
;p2   N p90 hard pulse
;p3   C p90 hard pulse
;p5   N p180 hard pulse
;p6   C p180 hard pulse
;p11  NCO CP contact time (11 ms)
;p13  C selective p180 duration
;p14 soft 180 carbon on res. (sqrt(3)/(2*delta w))
;p15 HN CP contact time
;p17 CAN TROP pulse length (3600us @ 55.555 kHz)
;p19 NCO TROP length (3600 us @55.555 kHz)
;p21 NH TROP length (792us @ 55.555 kHz)
;p22 water suppression pulse length (10-25 ms)
;p28 Trim pulse on CO
;p29 BSHCP contact time (5-7 ms)

;pl1  H p90 power
;pl2  N p90 power
;pl3  C hard power level
;pl5  N- NH CP power
```

```
;pl12 H decoupling power
;pl13 C selective 180 (Q3) power
;pl14 soft rectangular power (pi/2 equivalent)
;pl16 water suppression pulse power (10-20 kHz)
;pl22 decoupling on N (10-25 kHz)
;pl23 decoupling on C (10-25 kHz)
;pl28 C NCO CP power (ca. 1/3 spinning)

;sp2 NH H TROP power (100 kHz)
;sp6 NCO N CP power (ca. 2/3 spinning)
;sp7 CAN CA TROP power (80 kHz)
;sp8 H HN CP power
;sp20 NH N TROP power (70 kHz)
;sp27 CAN N TROP power (55 kHz)
;sp12 C selective 180 power
;sp29 BSHCP power


;spnam2 TROP-NH-MAS55kHz44rp-chan1H.shp
;spnam6 Ramp100-80
;spnam7 TROP-CAN-MAS55kHz200rp-chan13C.shp
;spnam8 Tang75
;spnam20 TROP-NH-MAS55kHz44rp-chan15N.shp
;spnam27 TROP-CAN-MAS55kHz200rp-chan15N.shp
;spnam28 TROP-COCA-XXXMHz-MAS55kHz-99rp-chan13C.shp
;spnam29 Ramp100-90

;cpdprg1 H decoupling (low power)
;cpdprg2 WALTZ or similar
;cpdprg3 WALTZ or similar

;cnst19 offset for water suppression !!in Hz!!
;cnst10 offset for HN CP !!in Hz!!
;cnst31 CO offset in ppm (173 ppm)
;cnst30 carbon decoupling offset in ppm (110 ppm)
;cnst32 CA offset in ppm (55 ppm)

;============================
; Include file for Protection
;============================

#include <Avance.incl>
#include <Delay.incl>

;==============
; Set variables
;==============

"cnst63 = plw12"
"cnst62 = plw22"
"cnst61 = plw23"
"cnst60 = plw16"
"p6 = 2.0*p3"
"p5 = 2.0*p2"
"d11=30m"
"d0=0.2u"
"d10=0.2u"
"d20=0.2u"
"d30=0.2u"

"in0=inf1/2"  ;N+1
"in20=inf2/2" ;H+1
"in30=inf3/2" ;Ca
```

```
"in10=inf4/2" ;N

"spoffs12 = (cnst31-cnst32)*bf3/1000000"

;============================
; Protection for parameters
;============================
1m
   if "p1 > 100u"     goto Problem
   if "p2 > 100u"     goto Problem
   if "p3 > 100u"     goto Problem
   if "p28 >10u"     goto Problem
   if "p28 >1000u"    goto Problem
   if "p15> 3000u"    goto Problem
   if "p16> 2000u"    goto Problem
   if "p17> 11001u"    goto Problem
   if "p18> 11001u"    goto Problem
   if "p29> 7000u"    goto Problem
   if "p22 >20001u"   goto Problem
   if "aq > 55m"      goto Problem
   if "d1 < 0.3s"     goto Problem
   if "cnst63>0.2"     goto Problem
   if "cnst62>1.1"     goto Problem
   if "cnst61>0.1"     goto Problem
   if "cnst60>0.2"     goto Problem

  goto PassParams
  Problem, 1m
  print "Protection: Parameters not accepted, ending."
  goto HaltAcqu
  PassParams, 1m


;----- Relaxation & reset parameters -----

1 ze

2 d11
  40u do:f3
  40u do:f2
  40u do:f1     ;decouplers off

3 d1
  20u reset:f1     reset:f2  reset:f3
  20u fq=cnst10:f1 fq=cnst31(bf ppm):f3
  5u  pl1:f1       pl2:f2    pl3:f3

;----- 90 on H -----

  (p1 pl1 ph1):f1

;----- H (t1) evolution & N decoupling  -----

  1u pl22:f2 pl23:f3
  1u cpd2:f2 cpd3:f3
  d20*2.0
  2u do:f2 do:f3

;----- H/N CP -----

  (p15 pl5 ph13):f2  (p15:sp8 ph14):f1

;----- N (t2) evolution & C decoupling  -----
```

```
  2u  pl12:f1  pl3:f3
  2u  fq=cnst30(bf ppm):f3
  1u  cpd1:f1
  d0
 (p3 ph20):f3
 (p6 ph21):f3
 (p3 ph20):f3
  d0
  2u fq=cnst31(bf ppm):f3
  2u do:f1
  1u
;----- N/CO CP -----

  (p18 pl28 ph11):f3 (p18:sp6 ph10):f2

;----- Water suppression  -----
;----- N magnetization along Z  -----

  (p3 pl3 ph17):f3

  20u fq=cnst19:f1
      2u pl16:f1
  p22:f1 ph22
  p22:f1 ph23
  p22:f1 ph22
  p22:f1 ph23

  20u fq=0:f1
  20u fq=cnst10:f1


;----- N magnetization in XY plane  -----

  (p3 pl3 ph18):f3

;----- CO to CA BSHCP transfer  -----

  (p28 pl3 ph28):f3
  2u fq=cnst32(bf ppm):f3
  (p29:sp29 ph29):f3


;----- CA (t4) evolution & HC decoupling  -----
  2u pl12:f1 cpd1:f1
  1u
  d30
  (center (p13:sp12 ph20):f3 (p5 pl2 ph20):f2)
  d30
  1u
  2u do:f1
;----- Bloch - Siegert - shift compensation

       (p14 pl14 ph20):f3
       1u
       (center (p13:sp12 ph20):f3 (p5 pl2 ph20):f2)

;----- CA/N CP -----

       ; echo-antiecho determination for Ca
       (p3 pl3 ph7):f3            ; 1st half of 180
       (p3 pl3 ph8):f3            ; 2nd half of 180
       (p17:sp7 ph2):f3 (p17:sp27 ph16):f2
```

```
;----- N (t3) evolution & C decoupling  -----

  2u  pl12:f1  pl3:f3
  2u  fq=cnst31(bf ppm):f3
  2u  cpd1:f1
  d10
  (p3 ph20):f3
  (p6 ph21):f3
  (p3 ph20):f3
  d10
  6u do:f1

;----- N/H CP -----

; echo-antiecho determination
(p2 pl2 ph3):f2            ; 1st half of 180
(p2 pl2 ph4):f2            ; 2nd half of 180
2u fq=cnst10:f1 fq=cnst32(bf ppm):f3
(p21:sp20 ph12):f2 (p21:sp2 ph10):f1

 2u pl22:f2 pl23:f3
 2u cpd2:f2  cpd3:f3
 2u fq=0:f1

 go=2 ph31
 d11 do:f2 do:f3  mc #0 to 2

 F1PH(calph(ph13, +90.0), caldel(d0, +in0)) ;N i+1
 F2PH(calph(ph1, +90.0), caldel(d20, +in20)) ;H i+1
 F3EA(calph(ph8, +180.0), caldel(d30, +in30)) ;Ca i
 F4EA(calph(ph4, +180.0) & calph(ph8, +180.0), caldel(d10, +in10)) ;N i

HaltAcqu, 1m
exit

;----- Phase cycling -----

ph1=  3 3 3 3  3 3 3 3  1 1 1 1  1 1 1 1
ph2=  0

ph3 = 1
ph4 = 3
ph5 = 0
ph6 = 0

ph7 = 1
ph8 = 3

ph10= 1
ph11= 0 2
ph12= 0

ph13 = 1 1 1 1  3 3 3 3

ph14 = 0
ph16 = 0 0 2 2
ph17= 1
ph18= 3

ph20= 1
ph21= 2
ph22= 0
ph23= 1
```

```
ph29= 0
ph28 =1
ph31= 0 2 2 0  2 0 0 2  2 0 0 2  0 2 2 0

;=====================
; END Pulse program
;=====================
;; IMPORTANT NOTE
; On AV (avance) machines, its better to use "spf0" files instead of "sp0"
; The "sp0" files requires lot of time for power settings compared to "spf0"
```

Fully enhanced 4D hCOCANH

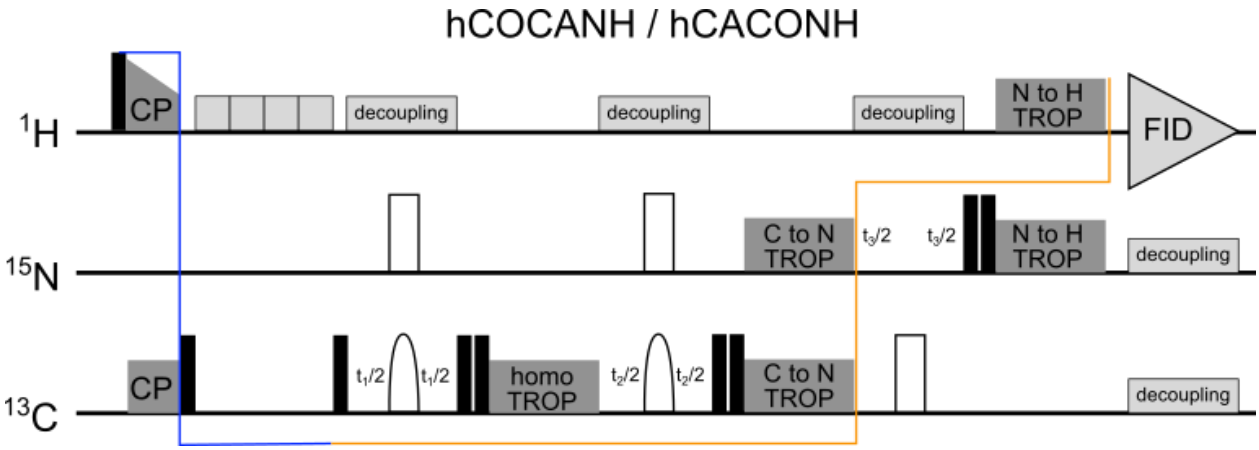

Fig. S6: Schematic representation of magnetization transfer steps in the fully enhanced hCACONH and hCOCANH experiment. The experiments only differ in the initial CP being either to Ca or CO, with the homoTROP respectively transferring from Ca to CO or CO to Ca. Blue represents normal transfers that do not yet carry both, x and y, components, while the orange line indicates transfers and dimensions which are sensitivity enhanced.

```
; sehCOCANH4D.alkl_v20240808
; 3-fold sensitivity-enhanced experiment using TROP pulses
; for COCA, CAN, and NH transfers

; for references see:
; 1. Blahut, J., et al. (2022). JACS, 144(38): 17336-17340.
; 2. Blahut, J., et al. (2023). JMR Open: 100122.
; 3. https://optimal-nmr.net/


; transfer is H->CO(F1)->CA(F2)->N(F3)->H(F4)
; f2 channel is Nitrogen
; f3 channel is Carbon


;=======================
; Variables introduction
;=======================

;p1   H p90 hard pulse
;p2   N p90 hard pulse
```

```
;p3   C p90 hard pulse
;p5   N p180 hard pulse
;p6   C p180 hard pulse
;p11  NCO CP contact time (11 ms)
;p13  C selective p180 duration
;p14 soft 180 carbon on res. (sqrt(3)/(2*delta w))
;p15 HCO CP contact time
;p17 CON TROP pulse length (3600us @ 55.555 kHz)
;p21 NH TROP length (792us @ 55.555 kHz)
;p22 water suppression pulse length (10-25 ms)
;p27 homoTROP length (1782us @ 55.555 kHz)

;pl1  H p90 power
;pl2  N p90 power
;pl3  C hard power level

;pl12 H decoupling power
;pl13 C selective 180 (Q3) power
;pl14 soft rectangular power (pi/2 equivalent)
;pl16 water suppression pulse power (10-20 kHz)
;pl22 decoupling on N (10-25 kHz)
;pl23 decoupling on C (10-25 kHz)
;pl27 CO HC CP power


;sp2 NH H TROP power (100 kHz)
;sp4 CON CO TROP power (80 kHz)
;sp6 HCA H CP power
;sp13 C selective 180 (Q3) power
;sp13 C selective 180 (Q3) power
;sp20 NH N TROP power (70 kHz)
;sp24 CON N TROP power (55 kHz)
;sp12 C selective 180 power
;sp28 CACO TROP power (75 kHz)


;spnam2 TROP-NH-MAS55kHz44rp-chan1H.shp
;spnam6 Tang75
;spnam7 TROP-CAN-MAS55kHz200rp-chan13C.shp
;spnam12 Q3 CO off reson.
;spnam13 Q3 Ca off reson.
;spnam20 TROP-NH-MAS55kHz44rp-chan15N.shp
;spnam27 TROP-CAN-MAS55kHz200rp-chan15N.shp
;spnam28 TROP-COCA-XXXMHz-MAS55kHz-99rp-chan13C.shp
;spnam29 Ramp100-90

;cpdprg1 H decoupling (low power)
;cpdprg2 WALTZ or similar
;cpdprg3 WALTZ or similar

;cnst19 offset for water suppression=O1P!!in ppm!!
;cnst10 offset for HN CP !!in ppm!!
;cnst31 CO offset in ppm (173 ppm)
;cnst30 carbon decoupling offset in ppm (110 ppm)
;cnst32 CA offset in ppm (55 ppm)

;============================
; Include file for Protection
;============================

#include <Avance.incl>
#include <Delay.incl>
```

```
;==============
; Set variables
;==============

"cnst63 = plw12"
"cnst62 = plw22"
"cnst61 = plw23"
"cnst60 = plw16"
"p6 = 2.0*p3"
"p5 = 2.0*p2"

"d11=30m"
"d0=0.2u"
"d10=0.2u"
"d20=0.2u"
"d30=0.2u"

"spoff13=bf3*((cnst32-cnst31)/1000000)"
"spoff12=bf3*((cnst31-cnst32)/1000000)"

"p27=99*18u"
"p21=44*18u"
"p17=200*18u"

"in20=inf1/2" ;CO
"in0=inf2/2"  ;CA
"in10=inf3/2" ;N


"d0=0.2u"
"d10=0.2u"
"d20=0.2u"

;==============================
; Protection for parameters
;==============================
1m
   if "p1 > 100u"     goto Problem
   if "p2 > 100u"     goto Problem
   if "p3 > 100u"     goto Problem
   if "p28 >10u"     goto Problem
   if "p28 >1000u"    goto Problem
   if "p15> 3000u"    goto Problem
   if "p16> 2000u"    goto Problem
   if "p17> 11001u"    goto Problem
   if "p18> 11001u"    goto Problem
   if "p29> 7000u"    goto Problem
   if "p22 >20001u"   goto Problem
   if "aq > 55m"      goto Problem
   if "d1 < 0.3s"     goto Problem
   if "cnst63>0.2"     goto Problem
   if "cnst62>1.1"     goto Problem
   if "cnst61>0.1"     goto Problem
   if "cnst60>0.2"     goto Problem

  goto PassParams
  Problem, 1m
  print "Protection: Parameters not accepted, ending."
  goto HaltAcqu
  PassParams, 1m


1 ze
```

```
2 d11
  40u do:f3
  40u do:f2
  40u do:f1     ;decouplers off

3 d1
  20u reset:f1     reset:f2  reset:f3
  20u fq=cnst10:f1 fq=cnst31(bf ppm):f3
  5u  pl1:f1       pl2:f2    pl3:f3
  2u fq=cnst32(bf ppm):f3     ; call cnst31 for ased. Set to CA carrier
  2u fq=cnst31(bf ppm):f3     ; switch to CO freq.


;----- 90 on H -----

  2u fq=cnst10(bf ppm):f1
  (p1 pl1 ph0):f1

;----- H/CO CP -----

  (p15:sp6 ph1):f1 (p15 pl27 ph2):f3

;----- Water suppression  -----
;----- C magnetization along Z  -----
  (p3 pl3 ph20):f3

  20u fq=cnst19(bf ppm):f1
  2u pl16:f1
  p22:f1 ph22
  p22:f1 ph23
       p22:f1 ph22
  p22:f1 ph23
  20u fq=cnst10(bf ppm):f1

;----- C magnetization in XY plane  -----

  (p3 pl3 ph21):f3

;----- CO (t1) evolution & decoupling  -----

  1u pl12:f1 cpds1:f1      ; 1H decoupling on
  1u pl13:f3
  d20
  (center (p2*2 pl2 ph11):f2 (p13:sp13 ph16):f3)
  d20

;----- Bloch - Siegert - shift compensation -----

  (p14 pl14 ph15):f3
  (p13:sp13 ph16):f3

  1u do:f1                  ; 1H decoupling off

;----- COCA transfer -----

  ; echo-antiecho determination for CO
  (p3 pl3 ph3):f3          ; 1st half of 180
  (p3 pl3 ph4):f3          ; 2nd half of 180
  (p27:sp28 ph27):f3


;----- CO (t2) evolution & decoupling  -----
```

```
  2u fq=cnst31(bf ppm):f3 ; switch to CO
  1u pl12:f1 cpds1:f1                   ; 1H decoupling on
  1u pl13:f3
  d0
  (center (p2*2 pl2 ph11):f2 (p13:sp12 ph16):f3)
  d0

;----- Bloch - Siegert - shift compensation -----

  (p14 pl14 ph15):f3
  1u pl13:f3
  (p13:sp12 ph16):f3
  1u do:f1

;----- CAN transfer -----

  ; echo-antiecho determination for CO
  (p3 pl3 ph17):f3    ; 1st half of 180
  (p3 pl3 ph18):f3    ; 2nd half of 180
  (p17:sp7 ph5):f3 (p17:sp27 ph6):f2

;----- N (t3) evolution & C decoupling  -----

  2u pl12:f1 cpds1:f1 fq=cnst30(bf ppm):f3
  d10
  (p3 pl3 ph12):f3
  (p3*2 pl3 ph23):f3
  (p3 pl3 ph12):f3
  d10
  2u do:f1

;----- N/H CP -----

  ; echo-antiecho determination
  (p2 pl2 ph7):f2   ; 1st half of 180
  (p2 pl2 ph8):f2   ; 2nd half of 180

  2u fq=cnst10(bf ppm):f1 fq=cnst32(bf ppm):f3
  (p21:sp20 ph9):f2 (p21:sp2 ph10):f1



1u pl22:f2 cpds2:f2
go=2 ph31
10m do:f2 do:f3 mc #0 to 2

F1EA(calph(ph4, +180), caldel(d20, +in20))
F2EA(calph(ph18, +180) & calph(ph4, +180),caldel(d0, +in0))
F3EA(calph(ph8, +180) & calph(ph18, +180) & calph(ph4, +360), caldel(d10, +in10))


HaltAcqu, 1m  jump address for protection files
exit

ph0=  1
ph1=  0
ph2=  1 3
ph20= 0 2
ph21= 2 0
ph11= 0
ph3=  0
ph4=  2
```

```
ph5=  0
ph6=  0 0 2 2
ph13= 0
ph12= 0
ph7=  0
ph8=  2
ph9=  0
ph10= 0       0 0 0  2 2 2 2
ph14= 0
ph15= 0
ph16= 0
ph22= 0
ph23= 1
ph27 = 0
ph29 = 3
ph28 = 2
ph17= 0
ph18= 2
ph24= 2
ph25= 3
ph31= 1 3 3 1  3 1 1 3
```

Fully enhanced 4D hCACONH

```
; sehCACONH4D.alkl_v20240807
; 3-fold sensitivity-enhanced experiment using TROP pulses
; for CACO, CON, and NH transfers

; for references see:
; 1. Blahut, J., et al. (2022). JACS, 144(38): 17336-17340.
; 2. Blahut, J., et al. (2023). JMR Open: 100122.
; 3. https://optimal-nmr.net/


; transfer is H->CA(F1)->CO(F2)->N(F3)->H(F4)
; f2 channel is Nitrogen
; f3 channel is Carbon


;=======================
; Variables introduction
;=======================

;p1   H p90 hard pulse
;p2   N p90 hard pulse
;p3   C p90 hard pulse
;p5   N p180 hard pulse
;p6   C p180 hard pulse
;p11  NCO CP contact time (11 ms)
;p13  C selective p180 duration
;p14 soft 180 carbon on res. (sqrt(3)/(2*delta w))
;p15 HCa CP contact time
;p17 CON TROP pulse length (3600us @ 55.555 kHz)
;p21 NH TROP length (792us @ 55.555 kHz)
;p22 water suppression pulse length (10-25 ms)
;p27 homoTROP length (1782us @ 55.555 kHz)

;pl1  H p90 power
;pl2  N p90 power
;pl3  C hard power level
```

```
;pl12 H decoupling power
;pl13 C selective 180 (Q3) power
;pl14 soft rectangular power (pi/2 equivalent)
;pl16 water suppression pulse power (10-20 kHz)
;pl22 decoupling on N (10-25 kHz)
;pl23 decoupling on C (10-25 kHz)
;pl27  Ca HC CP power


;sp2 NH H TROP power (100 kHz)
;sp4 CON CO TROP power (80 kHz)
;sp6 HCA H CP power
;sp13 C selective 180 (Q3) power
;sp13 C selective 180 (Q3) power
;sp20 NH N TROP power (70 kHz)
;sp24 CON N TROP power (55 kHz)
;sp12 C selective 180 power
;sp28 CACO TROP power (75 kHz)


;spnam2 TROP-NH-MAS55kHz44rp-chan1H.shp
;spnam6 Ramp100-80
;spnam4 TROP-CON-MAS55kHz200rp-chan13C.shp
;spnam8 Tang75
;spnam13 Q3 Ca off reson.
;spnam13 Q3 CO off reson.
;spnam20 TROP-NH-MAS55kHz44rp-chan15N.shp
;spnam24 TROP-CON-MAS55kHz200rp-chan15N.shp
;spnam28 TROP-CACO-XXXMHz-MAS55kHz-99rp-chan13C.shp
;spnam29 Ramp100-90

;cpdprg1 H decoupling (low power)
;cpdprg2 WALTZ or similar
;cpdprg3 WALTZ or similar

;cnst19 offset for water suppression=O1P!!in ppm!!
;cnst10 offset for HN CP !!in ppm!!
;cnst31 CO offset in ppm (173 ppm)
;cnst30 carbon decoupling offset in ppm (110 ppm)
;cnst32 CA offset in ppm (55 ppm)

;============================
; Include file for Protection
;============================

#include <Avance.incl>
#include <Delay.incl>

;==============
; Set variables
;==============

"cnst63 = plw12"
"cnst62 = plw22"
"cnst61 = plw23"
"cnst60 = plw16"
"p6 = 2.0*p3"
"p5 = 2.0*p2"
"spw2=spw8"
"d11=30m"
"d0=0.2u"
"d10=0.2u"
```

```
"d20=0.2u"
"d30=0.2u"


"spoff13=bf3*((cnst31-cnst32)/1000000)"
"spoff12=bf3*((cnst32-cnst31)/1000000)"

"p27=99*18u"
"p21=44*18u"
"p17=200*18u"

"in20=inf1/2" ;CA
"in0=inf2/2"  ;CO
"in10=inf3/2" ;N


"d0=0.2u"
"d10=0.2u"
"d20=0.2u"

;============================
; Protection for parameters
;============================
1m
   if "p1 > 100u"     goto Problem
   if "p2 > 100u"     goto Problem
   if "p3 > 100u"     goto Problem
   if "p28 >10u"     goto Problem
   if "p28 >1000u"    goto Problem
   if "p15> 3000u"    goto Problem
   if "p16> 2000u"    goto Problem
   if "p17> 11001u"    goto Problem
   if "p18> 11001u"    goto Problem
   if "p29> 7000u"    goto Problem
   if "p22 >20001u"   goto Problem
   if "aq > 55m"      goto Problem
   if "d1 < 0.3s"     goto Problem
   if "cnst63>0.2"     goto Problem
   if "cnst62>1.1"     goto Problem
   if "cnst61>0.1"     goto Problem
   if "cnst60>0.2"     goto Problem

  goto PassParams
  Problem, 1m
  print "Protection: Parameters not accepted, ending."
  goto HaltAcqu
  PassParams, 1m



1 ze

2 d11
  40u do:f3
  40u do:f2
  40u do:f1     ;decouplers off

3 d1
  20u reset:f1     reset:f2  reset:f3
  20u fq=cnst10:f1 fq=cnst31(bf ppm):f3
  5u  pl1:f1       pl2:f2    pl3:f3
  2u fq=cnst31(bf ppm):f3     ; call cnst31 for ased. Set to CO carrier
  2u fq=cnst32(bf ppm):f3     ; switch to Ca freq.
```

```
;----- 90 on H -----

  2u fq=cnst10(bf ppm):f1
  (p1 pl1 ph0):f1

;----- H/Ca CP -----

  (p15:sp6 ph1):f1 (p15 pl27 ph2):f3

;----- Water suppression  -----
;----- C magnetization along Z  -----
  (p3 pl3 ph20):f3

  20u fq=cnst19(bf ppm):f1
  2u pl16:f1
  p22:f1 ph22
  p22:f1 ph23
       p22:f1 ph22
  p22:f1 ph23
  20u fq=cnst10(bf ppm):f1

;----- C magnetization in XY plane  -----

  (p3 pl3 ph21):f3

;----- CA (t1) evolution & decoupling  -----

  1u pl12:f1 cpds1:f1      ; 1H decoupling on
  1u pl13:f3
  d20
  (center (p2*2 pl2 ph11):f2 (p13:sp13 ph16):f3)
  d20

;----- Bloch - Siegert - shift compensation -----

  (p14 pl14 ph15):f3
  (p13:sp13 ph16):f3

  1u do:f1                  ; 1H decoupling off

;----- CACO transfer -----

  ; echo-antiecho determination for Ca
  (p3 pl3 ph3):f3          ; 1st half of 180
  (p3 pl3 ph4):f3          ; 2nd half of 180
  (p27:sp28 ph27):f3


;----- CO (t2) evolution & decoupling  -----

  2u fq=cnst31(bf ppm):f3 ; switch to CO
  1u pl12:f1 cpds1:f1                   ; 1H decoupling on
  1u pl13:f3
  d0
  (center (p2*2 pl2 ph11):f2 (p13:sp12 ph16):f3)
  d0

;----- Bloch - Siegert - shift compensation -----

  (p14 pl14 ph15):f3
  1u pl13:f3
```

```
  (p13:sp12 ph16):f3
  1u do:f1

;----- CON transfer -----

  ; echo-antiecho determination for CO
  (p3 pl3 ph17):f3    ; 1st half of 180
  (p3 pl3 ph18):f3    ; 2nd half of 180
  (p17:sp4 ph5):f3 (p17:sp24 ph6):f2

;----- N (t3) evolution & C decoupling  -----

  2u pl12:f1 cpds1:f1 fq=cnst30(bf ppm):f3
  d10
  (p3 pl3 ph12):f3
  (p3*2 pl3 ph23):f3
  (p3 pl3 ph12):f3
  d10
  2u do:f1

;----- N/H CP -----

  ; echo-antiecho determination
  (p2 pl2 ph7):f2   ; 1st half of 180
  (p2 pl2 ph8):f2   ; 2nd half of 180

  2u fq=cnst10(bf ppm):f1 fq=cnst32(bf ppm):f3
  (p21:sp20 ph9):f2 (p21:sp2 ph10):f1



1u pl22:f2 cpds2:f2
go=2 ph31
10m do:f2 do:f3 mc #0 to 2

F1EA(calph(ph4, +180), caldel(d20, +in20))
F2EA(calph(ph18, +180) & calph(ph4, +180),caldel(d0, +in0))
F3EA(calph(ph8, +180) & calph(ph18, +180) & calph(ph4, +360), caldel(d10, +in10))


HaltAcqu, 1m  jump address for protection files
exit

ph0=  1
ph1=  0
ph2=  1 3
ph20= 0 2
ph21= 2 0
ph11= 0
ph3=  0
ph4=  2
ph5=  0
ph6=  0 0 2 2
ph13= 0
ph12= 0
ph7=  0
ph8=  2
ph9=  0
ph10= 0      0 0 0  2 2 2 2
ph14= 0
ph15= 0
ph16= 0
ph22= 0
```

```
ph23= 1
ph27 = 0
ph29 = 3
ph28 = 2
ph17= 0
ph18= 2
ph24= 2
ph25= 3
ph31= 1 3 3 1  3 1 1 3
```

2-fold enhanced 4D hcaCBCANH using INEPT for CBCA transfer

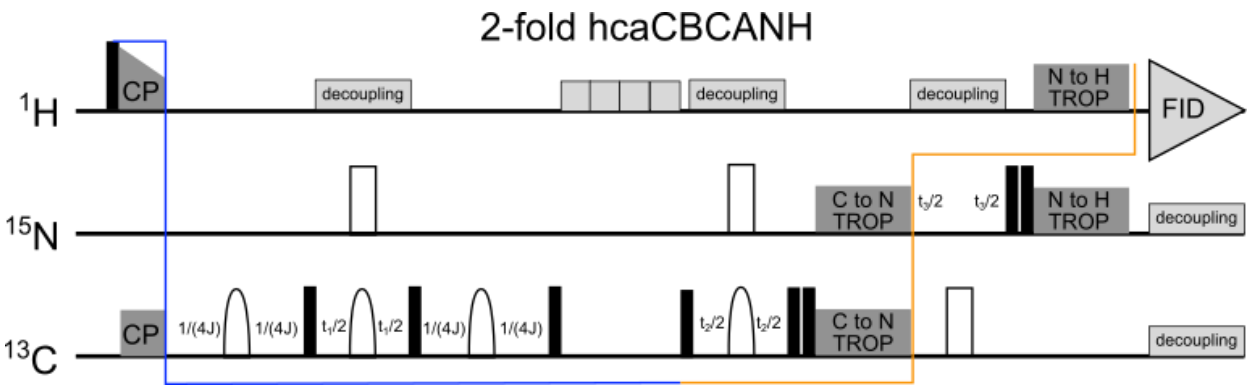

Fig. S7: Schematic representation of magnetization transfer steps in the doubly enhanced hcaCBCANH experiment. Blue represents normal transfers that do not yet carry both, x and y, components, while the orange line indicates transfers and dimensions which are sensitivity enhanced.

```
;sehCACBcaNH_INEPT_20250325.alkl
; 2-fold sensitivity-enhanced experiment using TROP pulses
; for CAN, and NH transfers and INEPT for CACB transfers

; for references see:
; 1. Blahut, J., et al. (2022). JACS, 144(38): 17336-17340.
; 2. Blahut, J., et al. (2023). JMR Open: 100122.
; 3. https://optimal-nmr.net/

; transfer is H->CA->CB(F1)->CA(F2)->N(F3)->H(F4)
; f2 channel is Nitrogen
; f3 channel is Carbon

;=======================
; Variables introduction
;=======================

;p1   H p90 hard pulse
;p2   N p90 hard pulse
;p3   C p90 hard pulse
;p5   N p180 hard pulse
```

```
;p6   C p180 hard pulse
;p13  C selective p180 duration
;p14 soft 180 carbon on res. (sqrt(3)/(2*delta w))
;p15 HCa CP contact time
;p17 CON TROP pulse length (3600us @ 55.555 kHz)
;p21 NH TROP length (792us @ 55.555 kHz)
;p22 water suppression pulse length (10-25 ms)


;pl1  H p90 power
;pl2  N p90 power
;pl3  C hard power level

;pl12 H decoupling power
;pl13 C selective 180 (Q3) power
;pl14 soft rectangular power (pi/2 equivalent)
;pl16 water suppression pulse power (10-20 kHz)
;pl22 decoupling on N (10-25 kHz)
;pl23 decoupling on C (10-25 kHz)
;pl27 Ca HC CP power


;sp2 NH H TROP power (100 kHz)
;sp4 CON CO TROP power (80 kHz)
;sp6 HCA H CP power
;sp13 C selective 180 (Q3) power
;sp20 NH N TROP power (70 kHz)
;sp24 CON N TROP power (55 kHz)
;sp12 C selective 180 power
;sp28 CACO TROP power (75 kHz)


;spnam2 TROP-NH-MAS55kHz44rp-chan1H.shp
;spnam6 Tang75
;spnam4 TROP-CON-MAS55kHz200rp-chan13C.shp.
;spnam13 Q3 CO off reson.
;spnam20 TROP-NH-MAS55kHz44rp-chan15N.shp
;spnam24 TROP-CON-MAS55kHz200rp-chan15N.shp
;spnam28 TROP-CACO-XXXMHz-MAS55kHz-99rp-chan13C.shp
;spnam29 Ramp100-90

;cpdprg1 H decoupling (low power)
;cpdprg2 WALTZ or similar
;cpdprg3 WALTZ or similar

;cnst19 offset for water suppression=O1P!!in ppm!!
;cnst10 offset for HN CP !!in ppm!!
;cnst31 CO offset in ppm (173 ppm)
;cnst30 carbon decoupling offset in ppm (110 ppm)
;cnst32 CA offset in ppm (55 ppm)

;d28: 1/(4J(CaCb) [3.6 msec]

;============================
; Include file for Protection
;============================

#include <Avance.incl>
#include <Delay.incl>

;==============
; Set variables
;==============
```

```
"cnst63 = plw12"
"cnst62 = plw22"
"cnst61 = plw23"
"cnst60 = plw16"
"p6 = 2.0*p3"
"p5 = 2.0*p2"
"d11=30m"
"d0=0.2u"
"d10=0.2u"
"d20=0.2u"


"in10=inf1/2" ;CB
"in0=inf2/2"  ;CA
"in20=inf3/2" ;N

"p21=44*18u"
"p17=200*18u"

"spoffs13 = (cnst31-cnst32)*bf3/1000000"

;============================
; Protection for parameters
;============================
1m
   if "p1 > 100u"     goto Problem
   if "p2 > 100u"     goto Problem
   if "p3 > 100u"     goto Problem
   if "p13 >1000u"    goto Problem
   if "p15> 3000u"    goto Problem
   if "p16> 2000u"    goto Problem
   if "p17> 11000u"    goto Problem
   if "p18> 11000u"    goto Problem
   if "p22 >20000u"   goto Problem
   if "aq > 80m"      goto Problem
   if "d1 < 0.44s"     goto Problem
   if "cnst63<26"     goto Problem
   if "cnst62<30"     goto Problem
   if "cnst61<30"     goto Problem
   if "cnst60<27"     goto Problem

   goto PassParams
   Problem, 1m
   print "Protection: Parameters not accepted, ending."
   goto HaltAcqu
   PassParams, 1m


;----- Relaxation & reset parameters -----

1 ze

2 d11
  40u do:f3
  40u do:f2
  40u do:f1     ;decouplers off

3 d1
  20u reset:f1     reset:f2  reset:f3
  20u fq=cnst10(bf ppm):f1  fq=cnst32(bf ppm):f3
  5u  pl1:f1       pl2:f2    pl27:f3
```

```
;----- 90 on H -----

  (p1 pl1 ph1):f1

;----- H/CA CP -----

  (p15 pl27 ph11):f3 (p15:sp6 ph10):f1

;----- CA/CB INEPT  -----

 ;1u pl12:f1
 ;1u cpd1:f1
 d28     ;1/(4JCaCb)
 (p14 pl14 ph2):f3
 d28

;----- transfer on CB, t2 evolution and CO decoupling -----

 (p3 pl3 ph19):f3
 2u fq=cnst13(bf ppm):f3
 d10
 (center (p13:sp13 ph20):f3  (p5 pl2 ph20):f2)
 d10
 2u

;----- (selective Ca and CO pulses)-----

  (p14 pl14 ph20):f3
  (center (p13:sp13 ph20):f3 (p5 ph20):f2)

;----- tansfer back on CA -----

  (p3 pl3 ph2):f3
  d28        ;1/(4JCaCb)
  (p14 pl14 ph2):f3
  d28
  2u fq=cnst32(bf ppm):f3

;----- C magnetization along Z  -----

  (p3 pl3 ph17):f3

;----- Water suppression  -----

  20u fq=cnst19(bf ppm):f1
  2u pl16:f1

     p22:f1 ph22
     p22:f1 ph23
     p22:f1 ph22
     p22:f1 ph23

 20u fq=cnst10(bf ppm):f1

;----- C magnetization in XY plane  -----

 (p3 pl3 ph18):f3

;---- CA evolution & decoupling -----

  d0
  (center (p13:sp13 ph20):f3 (p5 pl2 ph20):f2)
  d0
```

```
;----- (selective Ca and CO pulses)-----

 (p14 pl14 ph20):f3
 (center (p13:sp13 ph20):f3 (p5 ph20):f2)

;----- CA/N CP -----

; echo-antiecho determination
(p3 pl3 ph15):f3    ; 1st half of 180
(p3 pl3 ph16):f3    ; 2nd half of 180

(p32:sp4 ph7):f3 (p32:sp24 ph2):f2

;----- N (t2) evolution & C decoupling  -----

  1u  pl12:f1  pl3:f3
  2u  fq=cnst30(bf ppm):f3
  1u  cpd1:f1
  d20
 (p3 ph20):f3
 (p6 ph21):f3
 (p3 ph20):f3
  d20
  3u do:f1

;----- N/H transfer-----

; echo-antiecho determination
(p2 pl2 ph13):f2    ; 1st half of 180
(p2 pl2 ph14):f2    ; 2nd half of 180

(p21:sp20 ph12):f2 (p21:sp2 ph2):f1

 1u pl22:f2
 1u cpd2:f2

 go=2 ph31
 d11 do:f2  mc #0 to 2

 F1PH(calph(ph19, +90.0) & calph(ph11, +90.0), caldel(d10, +in10))
 F2EA(calph(ph16, +180),caldel(d0, +in0))
 F3EA(calph(ph14, +180.0) & calph(ph16, +180.0),caldel(d20, +in20))

 HaltAcqu, 1m
 exit
;----- Phase cycling -----

ph1=  0 2
ph2=  0
ph3=  0
ph4 = 0
ph5 = 0
ph6 = 0
ph7 = 0

ph10= 1
ph11= 0 0 2 2
ph12= 0

ph13 = 1
ph14 = 3
```

```
ph15 = 1
ph16 = 3

ph17= 1
ph18= 3

ph19 = 0

ph20= 1
ph21= 2
ph22= 0
ph23= 1
ph24 = 2
ph25 = 3
ph31= 0 2 2 0

;=====================
; END Pulse program
;=====================
;; IMPORTANT NOTE
; On AV (avance) machines, its better to use "spf0" files instead of "sp0"
; The "sp0" files requires lot of time for power settings compared to "spf0"
```

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